DR/2400
Spectrophotometer
PROCEDURE MANUAL

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Section 1 Introduction

1.1 Abbreviations Used in this Manual

The following abbreviations are used throughout the text of the procedure section:

Table 1

Abbreviation	Definition	Abbreviation	Definition
°C	degree(s) Celsius (Centigrade)	**HR**	high range
°F	degree(s) Fahrenheit	**L**	liter—volume equal to one cubic decimeter (dm^3)
ACS	American Chemical Society reagent grade purity	**LR**	low range
APHA Standard Methods	*Standard Methods for the Examination of Water and Wastewater,* published jointly by the American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF), is the standard reference work for water analysis. Order from Hach requesting Cat. No. 22708-00, or from the Publication Office of the APHA. Many procedures contained in this manual are based on *Standard Methods.*	**MDL**	method detection limit
		MDB	marked dropping bottle
		mg/L	milligrams per liter (ppm)
		µg/L	micrograms per liter (ppb)
		mL	milliliter—1/1000 of a liter. It is approximately the same as a cubic centimeter (and is sometimes called a “cc”).
		MR	medium range
AV	AccuVac®	**NIPDWR**	National Interim Primary Drinking Water Regulations
Bicn	bicinchoninate	**NPDES**	National Pollutant Discharge Elimination System
conc	concentrated	**P**	phosphorus
DB	dropping bottle	**PCB**	poly chlorinated biphenyl
DBP	disinfection by-products	**ppb**	parts per billion
CFR	Code of Federal Regulations	**ppm**	parts per million
EDL	Estimated detection limit	**RL**	Rapid Liquid™
EPA	Environmental Protection Agency	**SCDB**	self-contained dropping bottle
F&T	free and total	**THM**	trihalomethane
FM	FerroMo®	**TNT**	Test ‘N Tube™
FV	FerroVer®	**TOC**	total organic carbon
FZ	FerroZine®	**TPH**	total petroleum hydrocarbons
g	grams	**TPTZ**	2,4,6-Tri-(2-Pyridyl)-1,3,5-Triazine
gr/gal	grains per gallon (1 gr/gal = 17.12 mg/L)	**USEPA**	United States Environmental Protection Agency
		ULR	ultra low range

1.2 Conversions

1.2.1 Chemical Species

1.2.2 Hardness

Table 2 lists the factors for converting hardness from one unit of measure to another. For example, to convert mg/L $CaCO_3$ to German parts/100,000 CaO, multiply the value in mg/L x 0.056**.**

Table 2 Hardness Conversion Factors

Units of Measure	mg/L $CaCO_3$	British gr/gal (Imperial) $CaCO_3$	American gr/gal (US) $CaCO_3$	French Parts/ 100,000 $CaCO_3$	German Parts/ 100,000 CaO	meq/L[1]	g/L CaO	lbs./cu ft $CaCO_3$
mg/L $CaCO_3$	1.0	0.07	0.058	0.1	0.056	0.02	$5.6x10^{-4}$	$6.23x10^{-5}$
English gr/gal $CaCO_3$	14.3	1.0	0.83	1.43	0.83	0.286	$8.0x10^{-3}$	$8.9x10^{-4}$
US gr/gal $CaCO_3$	17.1	1.2	1.0	1.72	0.96	0.343	$9.66x10^{-3}$	$1.07x10^{-3}$
Fr. p/ 100,000 $CaCO_3$	10.0	0.7	0.58	1.0	0.56	0.2	$5.6x10^{-3}$	$6.23x10^{-4}$
Ger. p/100,000 CaO	17.9	1.25	1.04	1.79	1.0	0.358	$1x10^{-2}$	$1.12x10^{-3}$
meq/L	50.0	3.5	2.9	5.0	2.8	1.0	$2.8x10^{-2}$	$3.11x10^{-2}$
g/L CaO	1790.0	125.0	104.2	179.0	100.0	35.8	1.0	0.112
lbs./cu ft $CaCO_3$	16,100.0	1,123.0	935.0	1,610.0	900.0	321.0	9.0	1.0

[1]epm/L, or mval/L

Note: $\frac{meq}{L} = N \bullet 1000$

Section 2 Laboratory Practices

2.1 Temperature

Most tests in this manual perform most accurately when the sample temperature is between 20 °C and 25 °C (68 to 77 °F). A note in the individual procedure will state any special temperature requirements.

2.2 Mixing

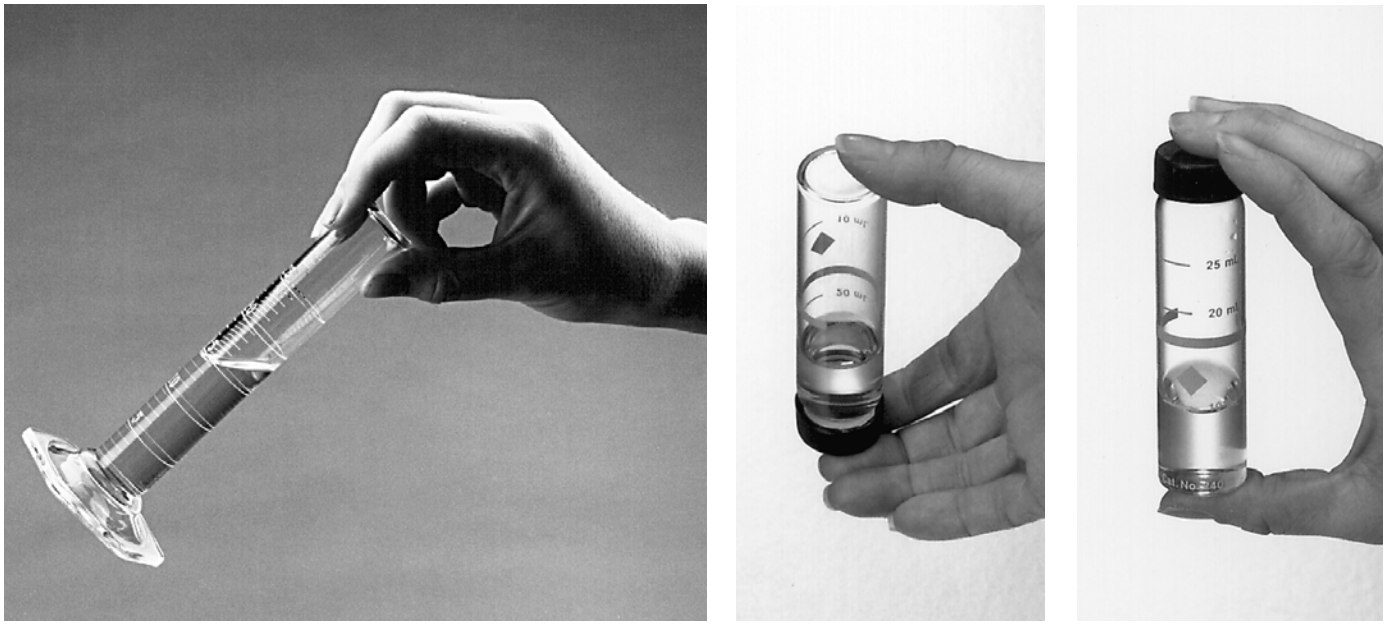
Swirling is recommended when mixing samples in a graduated cylinder or a titration flask. Grip the cylinder (or flask) firmly with the tips of the thumb and first two fingers (see *Figure 1*). Hold the cylinder at a 45-degree angle and make a circling motion from your wrist. This should move the cylinder in an approximately 12-inch circle, creating enough rotation to complete the mixing in a few turns.

Swirling is the gentlest method of mixing and offers the least chance for atmospheric contamination when testing for carbon dioxide and other gases.

Inverting allows for thorough mixing in a capped sample cell or a mixing cylinder. Hold the cell, or cylinder, in a vertical position with the cap on top. Invert, so that the cap is on the bottom. Return it to its original position. (See *Figure 1*.) This may be repeated as needed.

Both methods are simple, but take a bit of practice to get the best results.

Figure 1 Swirling a Cylinder and Inverting a Sample Cell



2.3 Digestion

Several procedures require sample digestion. Digestion uses chemicals and heat to break down a substance into components that can be analyzed. This section describes three different digestion procedures.

The Hach Digesdahl system is a process that yields a digest suitable for the determination of metals, total phosphorus and total Kjeldahl nitrogen (TKN). It is rapid, convenient, and the method of choice.

For USEPA reporting purposes, USEPA-approved digestions are required. USEPA presents two digestions (mild and vigorous) for metals analysis. These are much more inconvenient and time consuming compared to the Hach Digesdahl system. Other digestion procedures are required for mercury, arsenic, phosphorus and TKN.

See *Section 4 Sample Pretreatment by Digestion* for more on sample digestion.

2.3.1 EPA Mild Digestion with Hot Plate for Metals Analysis Only

1. Acidify the entire sample at the time of collection with concentrated nitric acid by adding 5 mL of acid per liter (or quart) of sample.
2. Transfer 100 mL of well-mixed sample to a beaker or flask. Add 5 mL of distilled 1:1 hydrochloric acid (HCl).
3. Heat using a steam bath or hot plate until the volume has been reduced to 15–20 mL. Make certain the sample does not boil.
4. After this treatment, the sample may be filtered to remove any insoluble material.
5. Adjust the digested sample to pH 4 by drop-wise addition of 5.0 N Sodium Hydroxide Standard Solution. Mix thoroughly and check the pH after each addition.
6. Quantitatively transfer the sample with deionized water to a 100-mL volumetric flask and dilute to volume with deionized water. Continue with the procedure. This mild digestion may not suffice for all sample types. A reagent blank also should be carried through the digestion and measurement procedures.

2.3.2 EPA Vigorous Digestion with Hot Plate for Metals Analysis Only

A vigorous digestion can be followed to ensure all organo-metallic bonds are broken.

1. Acidify the entire sample with redistilled 1:1 Nitric Acid Solution to a pH of less than two. Do not filter the sample before digestion.
2. Transfer an appropriate sample volume (see *Table 3*) into a beaker and add 3 mL of concentrated redistilled nitric acid.
3. Place the beaker on a hot plate and evaporate to near dryness, making certain the sample does not boil.
4. Cool the beaker and add another 3 mL of the concentrated redistilled nitric acid.
5. Cover the beaker with a watch glass and return it to the hot plate. Increase the temperature of the hot plate so that a gentle reflux occurs. Add additional acid, if necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change color or appearance with continued refluxing).
6. Again, evaporate to near dryness (do not bake) and cool the beaker. If any residue or precipitate results from the evaporation, add redistilled 1:1 hydrochloric acid (5 mL per 100 mL of final volume). See *Table 3*.

7. Warm the beaker. Add 5 mL of 5.0 N sodium hydroxide and quantitatively transfer the sample with deionized water to a volumetric flask. See *Table 3* below for the suggested final volume.
8. Adjust the sample to pH 4 by drop-wise addition of 5.0 N Sodium Hydroxide Standard Solution; mix thoroughly and check the pH after each addition. Dilute to volume with deionized water. Multiply the result by the correction factor in *Table 3*. A reagent blank also should be carried through the digestion and measurement procedures.

Table 3 Vigorous Digestion Volumes

Expected Metal Concentration	Suggested Sample Vol. for Digestion	Suggested Volume of 1:1 HCl	Suggested Final Volume After Digestion	Correction Factor
1 mg/L	50 mL	10 mL	200 mL	4
10 mg/L	5 mL	10 mL	200 mL	40
100 mg/L	1 mL	25 mL	500 mL	500

2.3.3 General Digesdahl Digestion (Not USEPA accepted)

Many samples may be digested using the Digesdahl Digestion Apparatus (Cat. No. 23130). It is designed to digest many types of samples such as oils, wastewater, sludges, feeds, grains, plating baths, food, and soils. In this procedure the sample is oxidized by a mixture of sulfuric acid and hydrogen peroxide. Digestion of a dry sample requires less than ten minutes, while liquid samples require about 1 minute/mL. The digestion is done in a special flat-bottomed 100-mL volumetric flask. Aliquots (sample portions) are taken for analysis using colorimetric methods.

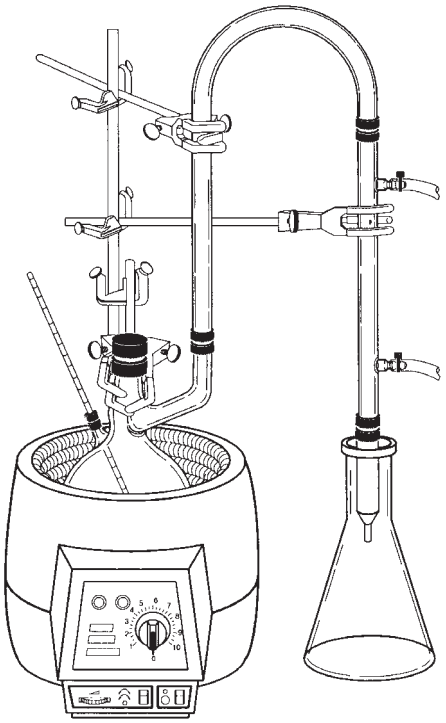
Procedures for digestion and using the Digesdahl Digestion Apparatus are based on the type and form of the sample, and are found in the *Digesdahl Digestion Apparatus Instruction Manual*, which is included with each Digesdahl Digestion Apparatus.

2.4 Distillation

Distillation is an effective, easy, and safe method of separating some chemical components for analysis. Hach Company offers the following equipment for distillation: the General Purpose Distillation Apparatus (Cat. No. 22653-00), shown in *Figure 2*; the Arsenic Distillation Apparatus Set (Cat. No. 22654-00); the Cyanide Distillation Apparatus Set (Cat. No. 22658-00); and the General Purpose Heater and Support Apparatus, which comes in a 115 VAC, 60 Hz model (Cat. No. 22744-00) and a 230 VAC, 50 Hz model (Cat. No. 22744-02).

The Hach Distillation Apparatus is suitable for water and wastewater that requires sample pretreatment by distillation. Applications for the General Purpose Apparatus include: fluoride, albuminoid nitrogen, ammonia nitrogen, phenols, selenium, and volatile acids. The General Purpose Heater and Support Apparatus provides efficient heating and anchoring of the glassware.

Figure 2 General Purpose Distillation Apparatus



2.5 Filtration

Filtration separates particulates from an aqueous sample. It uses a porous medium that retains particulates but allows liquids to pass through. It is useful for removing turbidity (which may interfere in colorimetric analyses) from water samples.

Two methods of filtration are most frequently used: vacuum filtration and gravity filtration.

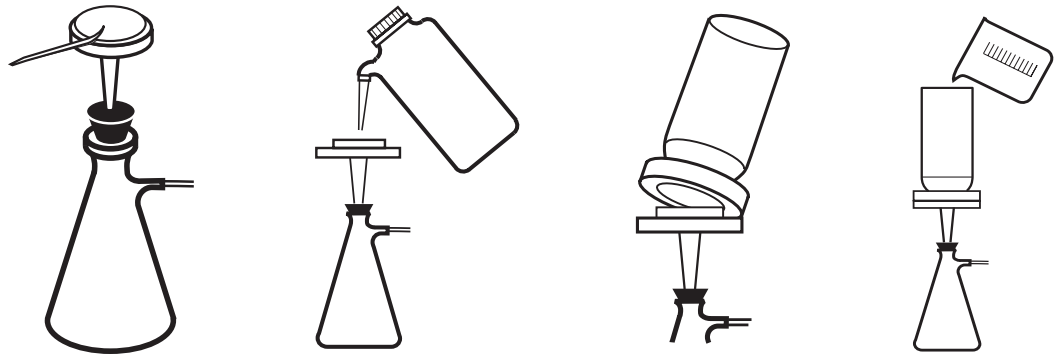
2.5.1 Vacuum Filtration

Vacuum filtration uses both suction and gravity to draw the liquid through the filter. An aspirator or vacuum pump is used to create suction (see *Figure 3*). It is faster than gravity filtration.

To filter using a vacuum:

1. Use tweezers to place a filter paper into the filter holder.
2. Place the filter holder assembly in the filtering flask. Dampen the filter with deionized water to ensure adhesion to the holder.
3. Position the funnel housing on the filter holder assembly.
4. While applying a vacuum to the filtering flask, transfer the sample to the filtering apparatus.
5. Slowly release the vacuum from the filtering flask and transfer the solution from the filter flask to another container.

Figure 3 Vacuum Filtration



Required Apparatus for Vacuum Filtration

Description	Unit	Cat. No.
Filter Discs, glass fiber, 47-mm	100/pkg	2530-00
Filter Holder, membrane, 47-mm	each	13529-00
Flask, filtering, 500-mL	each	546-49
Pump, vacuum, hand operated	each	28248-00
or		
Pump, vacuum, portable, 115 VAC	each	14697-00
Pump, vacuum, portable, 230 VAC	each	28248-01

2.5.2 Gravity Filtration

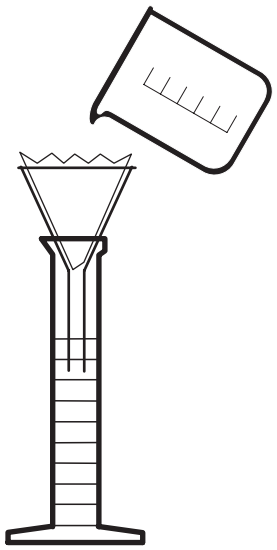
Many of the procedures in this manual use gravity filtration. This requires only filter paper, a conical funnel, and a receiving flask (see *Figure 4*). Gravity filtration is better for retaining fine particles. The rate of filtration increases as the volume increases in the filter cone, but never fill the cone more than three-quarters full.

Note: *Pretreatment using acid and heat is often necessary for metals testing. Filter paper will not withstand such an environment; therefore, vacuum filtration with glass fiber filter discs is recommended. Also, glass filter discs do not retain colored species like the paper filters do.*

To filter using gravity:

1. Place a folded filter paper into the funnel.
2. Dampen the filter paper with deionized water to adhere it to the funnel.
3. Place the funnel into an Erlenmeyer flask or graduated cylinder.
4. Pour the sample into the funnel.

Figure 4 Gravity Filtration



Required Apparatus for Gravity Filtration

Description	Unit	Cat No.
Cylinder, graduated, 100-mL	each	508-42
Funnel, poly, 65-mm	each	1083-67
Filter Paper, 12.5-cm, pleated	100/pkg	1894-57
Flask, erlenmeyer, 125-mL	each	505-43

2.6 Reagents

2.6.1 Reagent and Standard Stability

Chemicals supplied with the DR/2400 Portable Spectrophotometer and Portable Laboratory have the maximum shelf life when stored in a location that is cool, dark, and dry. The product label will specify any special storage needs.

It is always good laboratory practice to date chemicals upon receipt and to rotate supplies so the older supplies are used first.

Absorption of moisture, carbon dioxide, or other gases from the atmosphere; bacterial action; high temperatures or light (with photosensitive compounds) may affect the reagent shelf life. In some cases, reaction with the storage container or interaction of reagent components may occur.

In its quest for reagent stability, Hach has developed many unique formulations, methods of analysis, and forms of packaging.

2.6.2 Reagent Blank

In several tests, the contribution of the reagent(s) to the final reading is of such a magnitude that it must be compensated for whenever the test is performed. The

term reagent blank refers to that portion of the test result contributed solely by the reagent. This produces a positive error in the test results.

Every effort is made to produce reagents with the lowest possible blank; and, for most reagents, it is less than 0.009 absorbance units. It is sometimes impossible or impractical to produce reagents with such a low blank. When using such reagents, it is best to determine the reagent blank by performing the procedure using high-quality water (deionized, distilled, etc.) to zero the instrument.The resulting value is then expressed in the concentration units of the test and is subtracted from each sample determination that uses the same reagent lot. The DR/2400 Spectrophotometer software allows the reagent blank value to be stored and subtracted automatically from each sample value (see *Running a Blank* in the DR/2400 instrument manual). The reagent blank needs to be determined only at first use, when a new lot of reagent has been opened, or if you suspect contamination has occurred.

In most tests, the reagent blank is so small the instrument may be zeroed on either an untreated portion of the original water sample or on deionized water. This will not result in a significant loss of accuracy unless the test is for very low levels of a chemical. It is then best to use a reagent blank prepared as above.

2.7 Sample Dilution

Most Hach colorimetric tests use volumes of 10 and 25 mL. However, in some tests, the color developed in the sample may be too intense to be measured due to high levels of analyte, or unexpected colors may develop due to an interference. In either case, dilute the sample to produce a measurable endpoint or to determine if interfering substances are present.

To dilute the sample easily, pipet the chosen sample portion into a clean graduated cylinder (or volumetric flask for more accurate work). Fill the cylinder (or flask) to the desired volume with deionized water. Mix well. Use the diluted sample when running the test.

Table 4 shows relative quantities and the multiplication factors to use with a graduated cylinder. The concentration of the sample is equal to the diluted sample result multiplied by the multiplication factor.

Table 4 Sample Dilution Volumes

Sample Volume (mL)	mL Deionized Water Used to Bring the Volume to 25 mL	Multiplication Factor
25.0	0.0	1
12.5	12.5	2
10.0[1]	15.0	2.5
5.0*	20.0	5
2.5*	22.5	10
1.0*	24.0	25
0.250*	24.75	100

[1]For sample sizes of 10 mL or less, use a pipet to measure the sample into the graduated cylinder or volumetric flask.

More accurate dilutions can be done with a pipet and a 100-mL volumetric flask (see *Table 5* for more information). Pipet the sample and dilute to volume with deionized water. Swirl to mix.

Table 5 Multiplication Factors for Diluting to 100 mL

Sample Volume (mL)	Multiplication Factor
1	100
2	50
5	20
10	10
25	4
50	2

2.7.1 Sample Dilution with Interfering Substances

Sample dilution may influence the level at which a substance interferes. The effect of the interferences decreases as the dilution increases. In other words, higher levels of an interfering substance can be tolerated in the original sample if it is diluted before analysis.

An Example:

Copper does not interfere at or below 100 mg/L for a 25 mL sample in a procedure. If the sample volume is diluted with an equal volume of water, what is the level at which copper will not interfere?

$$\frac{\text{Total volume}}{\text{Sample volume}} = \text{Dilution factor}$$

$$\frac{25}{12.5} = 2$$

$$\text{Interference Level} \times \text{Dilution Factor} = \text{Interference level in sample}$$

$$100 \times 2 = 200$$

The level at which copper will not interfere in the diluted sample is at or below 200 mg/L.

2.8 AccuVac® Ampuls

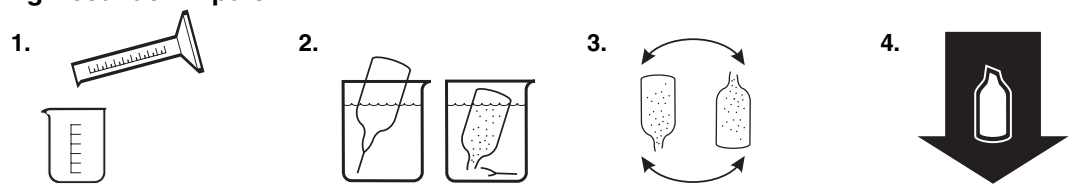
AccuVac Ampuls contain pre-measured powder or liquid vacuum-packed in optical-quality glass ampules.

To use AccuVac Ampuls:

1. Collect the sample in a beaker or other open container.
2. Place the ampule tip well below the sample surface and break the tip off (see *Figure 5*) against the beaker wall. The break must be far enough below the surface to prevent air from being drawn in as the level of the sample drops (the AccuVac Breaker may be used instead of breaking the ampule against the beaker side).

3. Invert the ampule several times to dissolve the reagent. Do not place your finger over the broken end; the liquid will stay in the ampule when inverted. Wipe the ampule with a lint-free cloth to remove fingerprints, etc.
4. Insert the ampule into the sample cell holder in the DR/2400 Spectrophotometer instrument and read the results directly.

Figure 5 Using AccuVac Ampuls

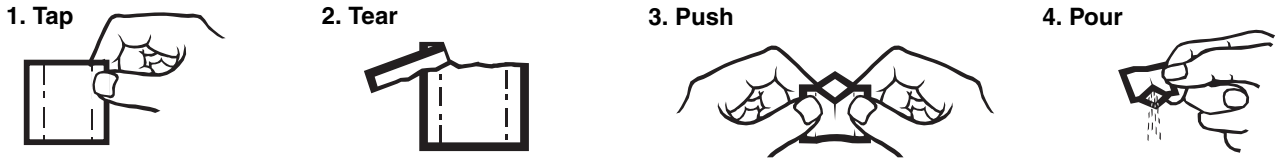


2.9 PermaChem® Pillows

To use PermaChem pillows (see *Figure 6*):

1. Tap the pillow on a hard surface to collect the powdered reagent in the bottom.
2. Tear (or cut) across, holding the pillow away from your face.
3. Using two hands, push both sides toward each other to form a spout.
4. Pour the pillow contents into the sample cell and continue the procedure according to the instructions.

Figure 6 Opening PermaChem Pillows



2.10 Sample Cells

A set of 10-mL round sample cells are shipped with the DR/2400 Portable Spectrophotometer instrument. The same solution in both cells will give the same absorbance (within ±0.002 Abs). The same solution in both cells should give approximately the same absorbance. For more information, see *Section 2.10.4 Matching of Sample Cells*, below.

Note: *25-mL cells with the catalog number printed across the bottom cannot be used in the DR/2400.*

2.10.1 Orientation of Sample Cells

To minimize measurement variability when using a particular cell, always orient the cell in the same manner before placing it into the cell holder. The fill marks on the cells can be used as orientation guides for positioning the cells.

2.10.2 Care of Sample Cells

When not in use, store the sample cells in their boxes to protect them from scratches and breakage. After use, empty and clean the cells; avoid leaving colored solutions in the cells for extended periods of time.

2.10.3 Cleaning of Sample Cells

Most laboratory detergents can be used at recommended concentrations. Neutral detergents, such as Liquinox, are safer when regular cleaning is required. When using a detergent, you can clean faster by increasing the temperature, or by using an ultrasonic bath. Finish by rinsing a few times with deionized water and allow to air dry.

Cells may also be cleaned with acid, followed by rinsing thoroughly with deionized water.

Individual procedures may require special cleaning methods.

2.10.4 Matching of Sample Cells

Although sample cells shipped with the instrument are distortion-free, nicks and scratches from handling may cause an optical mismatch between two sample cells and introduce error into the test results. This type of error may be avoided by optically matching the sample cells as follows:

1. Turn the instrument on. In Instrument Setup, turn Display Lock **Off**.
2. Return to the Main Menu and select **Single Wavelength**.
3. Touch the λ key and select a wavelength of **510 nm,** or the wavelength to be used for the test.
4. Pour at least 25 mL (10 mL for 10-mL cells) of deionized water into each of two sample cells.
5. Place one sample cell into the cell holder. Orient the fill mark toward the front.
6. Touch **Zero**. The display will show: **0.000 Abs**
7. Place the other sample cell into the cell holder.
8. Wait about three seconds for the value to stabilize and read the absorbance. Record the resulting absorbance.
9. Turn the cell 180° and repeat *step 8*. Try to achieve an absorbance value within ±0.002 Abs of the first cell. Note the orientation of the cell.

If the sample cells cannot be matched within ±0.002 Abs, they may still be used by compensating for the difference. For example, if the second cell reads 0.003 absorbance units higher than the first cell, correct future readings (when using these two cells) by subtracting 0.003 absorbance units (or the equivalent concentration) from the reading. Likewise, if the second cell reads -0.003 absorbance units, add 0.003 absorbance units to the reading.

2.11 Other Apparatus

2.11.1 Boiling Aids

Boiling is required in some procedures. Under some conditions *bumping* may occur causing sample loss or injury. Bumping is caused by the sudden, almost explosive, conversion of water to steam as it is heated. Use of a boiling aid, such as Boiling Chips (Cat. No. 14835-31), reduces bumping.

Make sure the boiling aids will not contaminate the sample. Do not use boiling aids (except glass beads, Cat. No. 2596-00) more than once. Loosely covering the sample during boiling will prevent splashing, reduce the chances of contamination, and minimize sample loss.

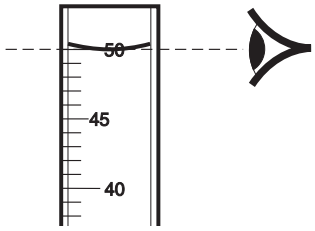
Individual procedures will recommend the specific boiling aid to use.

2.12 Achieving Accuracy in Volume Measurement

2.12.1 Pipets and Graduated Cylinders

When smaller sample quantities are used, the accuracy of measurements becomes increasingly important. *Figure 7* illustrates the proper way to read the sample level using the meniscus formed when the liquid wets the cylinder or pipet walls.

Figure 7 Reading the Meniscus



Before filling, rinse the pipet or cylinder two or three times with the sample to be tested. Use a pipet filler or pipet bulb to draw the sample into the pipet. ***Never pipet chemical reagent solutions or samples by mouth.*** When filling a pipet, keep the tip of the pipet below the surface of the sample as the sample is drawn into the pipet.

Serological pipets have marks that indicate the volume of liquid delivered by the pipet. The marks may extend to the tip of the pipet or may be only on the straight portion of the tube. If the marks are only on the straight part of the tube, fill serological pipets to the zero mark and discharge the sample by draining the sample until the meniscus is level with the desired mark. If the serological pipet has marks extending to the tip of the pipet, fill the pipet to the desired volume and drain all the sample from the pipet. Blow the sample out of the pipet tip for accurate measurements.

Volumetric (transfer) pipets have a bulb in the middle and a single ring above the bulb to indicate the volume of liquid when it is filled to the mark. To discharge a volumetric pipet, hold the tip of the pipet at a slight angle against the container wall and drain. **Do not** discharge the solution remaining in the tip of the pipet after draining. Volumetric pipets are designed to retain a small amount of sample in the pipet tip.

If droplets of sample cling to the walls of the pipet, the pipet is dirty and is not delivering the correct amount of sample. Wash the pipet thoroughly with a laboratory detergent or cleaning solution and rinse several times with deionized water.

2.12.2 The Pour-Thru Cell

The Pour-Thru Cell is an optional accessory that improves accuracy and makes measuring more convenient, especially when the analyte is present at low levels. Using the Pour-Thru Cell avoids any error that might result from optical differences between sample cells. See your *Instrument Manual* for instructions on installation. Not all procedures allow for using the Cell. The Pour-Thru Cell can be used with Hach chemistries provided a 25 mL sample is analyzed. Exceptions are noted in *Table 6*.

Table 6 Hach chemistries that cannot be used with the Pour-Thru cell

Aluminum ECR	Arsenic	Barium	Boron, Carmine
Cyanuric Acid	Fluoride	Formaldehyde	Lead, LeadTrak
Mercury	Nickel, Heptoxime	Nitrite, HR	PCB
Phenols	Potassium	Selenium	Silver
Suspended Solids	Sulfate	TPH	Volatile Acids
Zinc	Surfactants, Anionic (Detergents)		

The Pour-Thru Cell can be used with the following Hach chemistries, but special care must be taken to rinse the cell with deionized water between samples.

Table 7 Hach chemistries that require extra rinsing with the Pour-Thru cell

Aluminum, Aluminon	Chlorine Dioxide, LR	Cobalt, PAN
Copper, Porphyrin	Hardness, Calmagite	Manganese, LR, PAN
Nickel, PAN	Nitrate, MR	Nitrate, HR

The Pour-Thru Cell can also be used to run Nitrogen, Ammonia, Nessler Method, and TKN chemistries. Clean the cell by pouring a few sodium thiosulfate pentahydrate crystals into the cell. Rinse out the crystals with deionized water.

The Pour-Thru Cell **cannot** be used with 10-mL sample sizes and reagents. There is not sufficient volume to flush the cell completely; this leads to sample carryover errors. For some tests, you will find optional 25-mL reagents listed for use with the Pour-Thru Cell.

Instead of placing a 25-mL sample cell into the cell holder, you simply pour the solution into the funnel of the installed Pour-Thru Cell Assembly. Avoid spilling solution onto the instrument.

The funnel height and orientation may be adjusted. The funnel height determines the speed of sample flow through the cell. The higher the funnel, the faster the flow. To minimize air bubbles, adjust the funnel so that it drains completely with the final level of liquid in the tube about 5 cm (2 inches) below the tip of the funnel.

Take instrument readings after the solution has stopped flowing through the cell. Always rinse the cell thoroughly with deionized water after each series of tests, or as often as specified in the procedure.

Occasionally, remove the Pour-Thru Cell to check for any accumulation of film on the windows. If the windows appear hazy, soak the cell in a detergent bath and rinse thoroughly with deionized water.

Note: *The Cell cannot be used directly with Hach programs unless it is specified in the procedure. Using the Pour-Thru Cell with other Hach procedures requires a standard adjust or entering a dilution factor of 0.95. For more information, see Correcting for a Dilution Sample and Running a Standard Adjust in the DR/2400 Spectrophotometer Instrument Manual.*

Section 3 Chemical Analysis

3.1 Sample Collection, Preservation, and Storage

Correct sampling and storage are critical for accurate testing. Sampling devices and containers must be thoroughly clean to prevent carryover from previous samples. Preserve the sample properly; each procedure has information about sample preservation.

3.1.1 Collecting Water Samples

Use a clean container. Rinse the container several times with the water to be sampled before taking the sample. Document the location and procedure used for each sample taken. For example:

3.1.1.1 From a tap

Take samples as close as possible to the source of the supply. This lessens the influence of the distribution system on the sample. Let the water run long enough to flush the system. Fill sample containers slowly with a gentle stream to avoid turbulence and air bubbles.

When testing well water, let the pump run long enough to draw fresh groundwater into the system. Collect a sample from a tap near the well.

3.1.1.2 From open waters

Sample as near the middle of the body of water as is practical; at least several feet from the shore or edge of the tank.

Take the sample under the surface of the water. If you are using a capped container, submerge it before removing the cap.

3.1.1.3 Types of Containers

Table 8 lists recommended containers for specific parameters.

- **Polypropylene and Polyethylene —** These are the least expensive containers.
- **Quartz or TFE (tetrafluoroethylene, Teflon®)**—These are the best, and the most expensive.
- **Glass**—Glass provides a good general-purpose container. Avoid using soft-glass containers to collect samples for metals testing in the microgram-per-liter range.

  When determining silver, store samples in dark containers such as amber or brown glass.

Acid washing will thoroughly clean sample containers before use.

3.1.1.4 Acid Washing

If a procedure suggests acid washing, follow these steps:

a. Clean the glassware or plasticware with laboratory detergent. Phosphate-free detergent is best. (When determining phosphates, phosphate-free detergent *must* be used.)

b. Rinse well with tap water.

c. Rinse with a 1:1 hydrochloric acid solution or a 1:1 nitric acid solution. (Nitric acid is best when testing for lead or other metals.)

d. Rinse well with deionized water. For chromium, 12–15 rinses may be necessary. When determining ammonia and Kjeldahl nitrogen, the rinse water must be ammonia-free.

e. Air dry. Protect the glassware from fumes and other sources of contamination when storing.

Use chromic acid or chromium-free substitutes to remove organic deposits from glass containers. Afterward, rinse thoroughly with water to remove all traces of chromium.

Avoid introducing metal contaminants from containers, distilled water, or membrane filters.

3.1.1.5 Sample Splits

Samples must often be split or divided into separate containers for intra- or inter-laboratory use in studies, confirmation, alternative techniques, or maintaining additional sample for reference, or stability studies. It is very important that sample splits be done correctly.

- Collect a large volume of sample in a single container and transfer to smaller containers; do not fill the smaller containers individually from the water source.
- Thoroughly mix samples containing particulates or solids before splitting so that all the splits are homogeneous.
- If the sample requires filtering before analysis or storage, filter the entire sample before splitting.
- Use the same kind of container for all the splits.
- Analyze biologically active splits on the same day, or as close to the same day as is possible.
- Preserve all splits in the same way; if this is not done, the differing methods must be fully documented.
- When testing for volatile contaminants, fill containers samples to overflowing and cap carefully. Do not leave any headspace or air in the container.

3.1.2 Storage and Preservation

Because chemical and biological processes continue after collection, analyze the sample as soon as possible. This also reduces the chance for error and minimizes labor. When immediate analysis is not possible, the sample must be preserved. Preservation methods include pH control, chemical addition, refrigeration, and freezing.

Table 8 presents an overview of preservation methods and holding times for specific procedures.

You can preserve aluminum, cadmium, chromium, cobalt, copper, iron, lead, nickel, potassium, silver, and zinc samples for at least 24 hours by adding one Nitric Acid Solution Pillow 1:1 (Cat. No. 2540-98) per liter of sample. Check the pH with pH indicator paper or a pH meter to assure the pH is 2 or less. Add additional pillows if necessary. Adjust the sample pH prior to analysis by raising the pH to 4.5 with Sodium Hydroxide Standard Solution, 1 N or 5 N.

3.1.3 Correcting for Volume Additions

If you use a large volume of preservative or neutralizer, you must account for dilution by the acid added to preserve the sample, and/or the base used to adjust the pH to the range of the procedure. Make this correction as follows:

1. Determine the volume of initial sample, the volume of acid and base added, and the total final volume of the sample.
2. Divide the total volume by the initial volume.
3. Multiply the test result by this factor.

Example:

A one-liter sample was preserved with 2 mL of nitric acid. It was neutralized with 5 mL of 5 N sodium hydroxide. The result of the analysis procedure was 10.00 mg/L. What is the volume correction factor and correct result?

1. Total Volume = 1000 mL + 2 mL + 5 mL = 1007 mL
2. $\frac{1007}{1000}$ = 1.007 = volume correction factor
3. 10.0 mg/L × 1.007 = 10.07 mg/L = correct result

Hach 1:1 Nitric Acid Pillows contain 2.5 mL of acid: correct for this volume.

Table 8 Required Containers, Preservation Techniques and Holding Times[1]

Parameter No./Name	Container[2]	Preservation[3,4]	Maximum Holding Time[5]
Table 1A - Bacterial Tests			
1–4. Coliform, fecal and total	P,G	Cool, 4 °C, 0.008% $Na_2S_2O_3$	6 hours
5. Fecal streptococci	P,G	Cool, 4 °C, 0.008% $Na_2S_2O_3$	6 hours
Table 1A - Aquatic Toxicity Tests			
6–10 Toxicity, acute and chronic	P, G	Cool, 4 °C	36 hours
Table 1B - Chemical Tests			
1. Acidity	P, G	Cool, 4 °C	14 days
2. Alkalinity	P, G	Cool, 4 °C	14 days
4. Ammonia	P, G	Cool, 4 °C, H_2SO_4 to pH<2	28 days
9. Biochemical oxygen demand (BOD)	P, G	Cool, 4 °C	48 hours
10. Boron	P, PFTE or quartz	HNO_3 to pH<2	6 months
11. Bromide	P, G	None required	28 days
14. Biochemical oxygen demand, carbonaceous	P, G	Cool, 4 °C	48 hours
15. Chemical oxygen demand	P, G	Cool, 4 °C, H_2SO_4 to pH<2	28 days
16. Chloride	P, G	None required	28 days
17. Chlorine, total residual	P, G	None required	Analyze immediately
21. Color	P, G	Cool, 4 °C	48 hours
23–24. Cyanide, total and amenable to chlorination	P, G	Cool, 4 °C, NaOH to pH>12, 0.6 g ascorbic acid[6]	14 days[7]
25. Fluoride	P	None required	28 days

Table 8 Required Containers, Preservation Techniques and Holding Times[1] (continued)

Parameter No./Name	Container[2]	Preservation[3,4]	Maximum Holding Time[5]
27. Hardness	P, G	HNO_3 to pH<2, H_2SO_4 to pH<2	6 months
28. Hydrogen ion (pH)	P, G	None required	Analyze immediately
31, 43. Kjeldahl and organic nitrogen	P, G	Cool 4 °C, H_2SO_4 to pH<2	28 days
Metals[8]			
18. Chromium VI	P, G	Cool, 4 °C	24 hours
35. Mercury	P, G	HNO_3 to pH<2, Cool 4 °C	28 days
Metals, except boron, chromium VI and mercury [3, 5–8, 12, 13, 19, 20, 22, 26, 29, 30, 32–34, 36, 37, 45, 47, 51, 52, 58-60, 62, 63, 70-72, 74, 75][9].	P, G	HNO_3 to pH<2	6 months
38. Nitrate	P, G	Cool, 4 °C	48 hours
39. Nitrate-nitrite	P, G	Cool 4 °C, H_2SO_4 to pH<2	28 days
40. Nitrite	P, G	Cool, 4 °C	48 hours
41. Oil and grease	G	Cool, 4 °C, HCl or H_2SO_4 to pH<2	28 days
42. Organic Carbon	P, G	Cool, 4 °C, HCl or H_2SO4 or H_3PO_4 to pH<2	28 days
44. Orthophosphate	P, G	Filter immediately; Cool, 4 °C	48 hours
46. Oxygen, dissolved probe	G Bottle and top	None required	Analyze immediately
47. Winkler	G Bottle and top	Fix on site and store in dark	8 hours
48. Phenols	G only	Cool 4 °C, H_2SO_4 to pH<2	28 days
49. Phosphorus, elemental	G	Cool, 4 °C	48 hours
50. Phosphorus, total	P, G	Cool, 4 °C, H_2SO_4 to pH<2	28 days
53. Residue, Total	P, G	Cool, 4 °C	7 days
54. Residue, Filterable	P, G	Cool, 4 °C	7 days
55. Residue, Nonfilterable (TSS)	P, G	Cool, 4 °C	7 days
56. Residue, Settleable	P, G	Cool, 4 °C	48 hours
57. Residue, Volatile	P, G	Cool, 4 °C	7 days
61. Silica	P, PFTE or quartz	Cool, 4 °C	28 days
64. Specific Conductance	P, G	Cool, 4 °C	28 days
65. Sulfate	P, G	Cool, 4 °C	28 days
66. Sulfide	P, G	Cool 4 °C, add zinc acetate plus sodium hydroxide to pH>9	7 days
67. Sulfite	P, G	none required	Analyze immediately
68. Surfactants	P, G	Cool, 4 °C	48 hours
69. Temperature	P, G	None required	Analyze immediately
73. Turbidity	P, G	Cool, 4 °C	48 hours

[1]This table was taken from Table II published in the *Federal Register*, July 1, 1995, 40 CFR, Part 136.3, pages 643–645. Most organic tests are not included.

[2]Polyethylene (P) or glass (G), or PTFE Teflon

[3]Sample preservation should be performed immediately upon sample collection. For composite chemical samples each portion should be preserved at the time of collection. When use of an automated sampler makes it impossible to preserve each portion, then chemical samples may be preserved by maintaining at 4 °C until compositing and sample splitting is completed.

[4]When any sample is to be shipped by common carrier or sent through United States Mails, it must comply with the *Department of Transportation Hazardous Material Regulations* (49 CFR Part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO_3) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H_2SO_4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

[5]Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still be considered valid. Samples may be held for longer periods only if the permittee, or monitoring laboratory has data on file to show that the specific types of samples under study are stable for the longer time, and has received a variance from the Regional Administrator under §136.3(e). Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the sample for a shorter time if knowledge exists to show that this is necessary to maintain sample stability. See §136.3(e) for details. The term "analyze immediately" usually means within 15 minutes or less after sample collection.

[6]Should only be used in the presence of residual chlorine.

[7]Maximum holding time is 24 hours when sulfide is present. Optionally all samples may be tested with lead acetate paper before pH adjustments in order to determine if sulfide is present. If sulfide is present, it can be removed by the addition of cadmium nitrate powder until a negative spot test is obtained. The sample is filtered and then NaOH is added to pH 12.

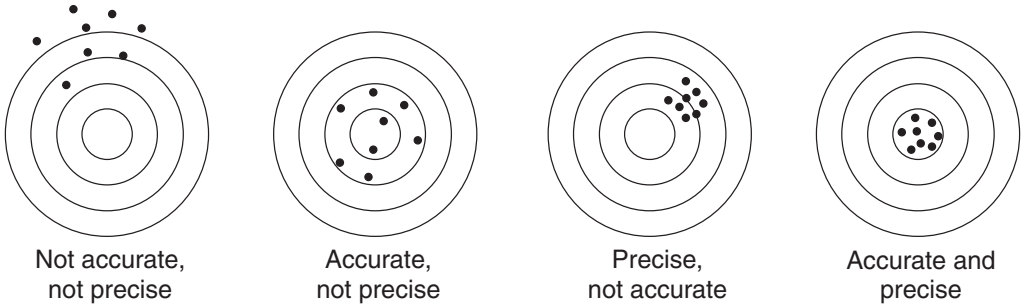
[8]Samples should be filtered immediately on-site before adding preservative for dissolved metals.

[9]Numbers refer to parameter numbers in 40 CFR, Part 136.3, Table 1B.

3.2 Checking for Accuracy and Precision

Accuracy defines the closeness of a test result to the true value. Precision defines the closeness of repeated measurements to each other. Although precise results suggest accuracy, they can be inaccurate. Both the accuracy and the precision of test results can be evaluated by using standard additions or standard solutions.

Figure 8 Precision and Accuracy Illustrated



3.2.1 Standard Solutions

A standard solution may be ordered as a prepared reagent or it may be made in the laboratory. It is a solution of a known composition and concentration. The accuracy of your instrument may be checked by using a standard solution in place of the sample water in a procedure.

3.2.2 Standard Additions

In general, this technique requires adding a small amount of a standard solution to the sample water and repeating the procedure. You should get close to 100% recovery. If not, a problem exists and must be identified.

1. To check for an interference, add the standard solution to deionized water and repeat the procedure. If you now get close to 100% recovery, you have an interference and must identify the source of the interference. If your recovery is still poor, use the following checklist:
   - Are you following the procedure exactly?
   - Are you using the proper reagents in the proper order and concentration?
   - Are you waiting the full time necessary for color development?
   - Are you using the correct glassware? Is it clean?
   - Does the test need a specific sample temperature?
   - Is the pH of the sample in the correct range?

   A careful reading of the procedure should help you to answer these questions.
2. Check the performance of your instrument. Follow the instructions in the *Maintenance* section of the *Instrument Manual*.
3. Check your reagents. Repeat the standard additions test using fresh reagents. It may also be necessary to replace the standard solution.

If you have still not located the problem, use the decision tree in *Figure 9*, and the explanations of each branch, for further help.

3.2.3 Explanation of the Standard Additions Decision Tree

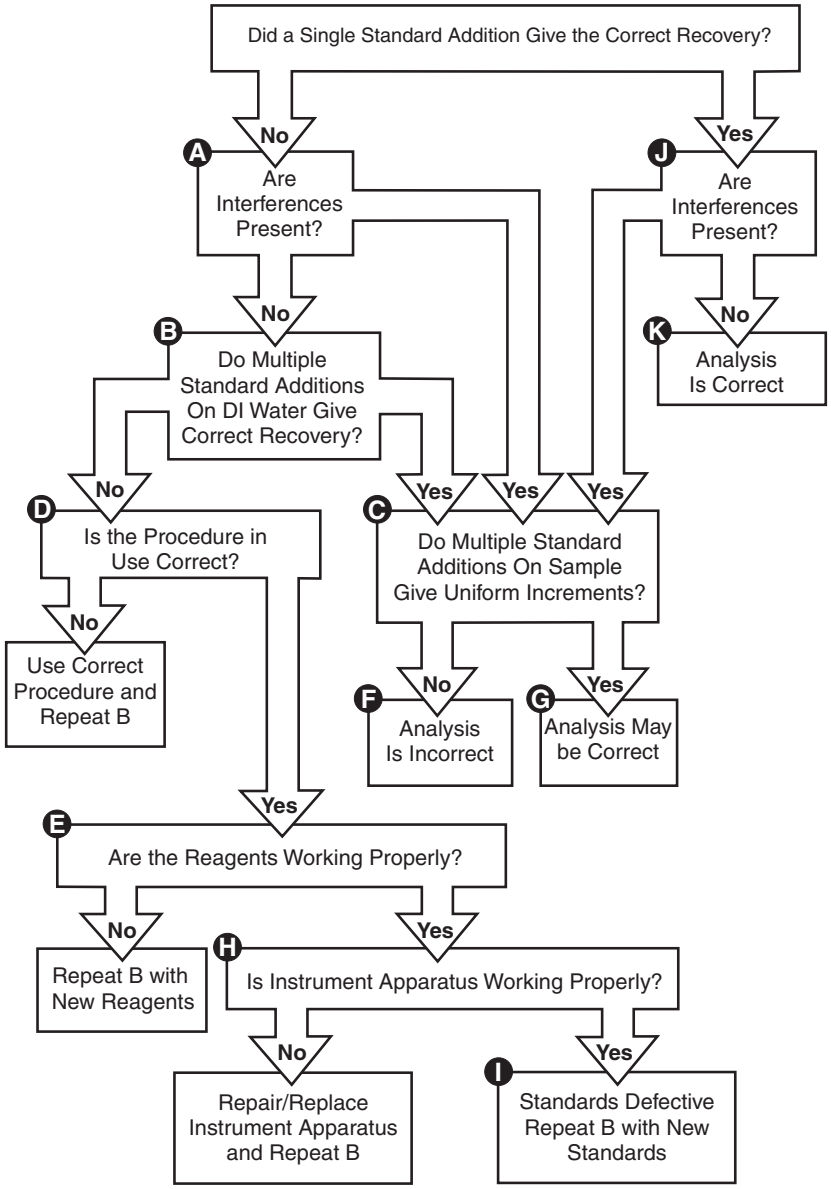
If a single standard addition to the sample does not give the expected increase in concentration, a likely cause may be the presence of interferences. (Other causes might be defective reagents, a defective instrument or apparatus, defective standard, or incorrect technique.)

Branch A

If interferences are known to be present, proceed to *Branch C*.

If interferences are known or assumed to be absent, look for other sources of error. Proceed to *Branch B*.

Figure 9 Standard Additions Decision Tree



Branch B Perform multiple standard additions on a sample of deionized water as follows:

Note: *For this example, a 25 mL sample size and a 50 mg/L iron standard is used.*

1. Pour 25 mL of deionized water into a 25-mL sample cell.
2. Add 0.1 mL of the appropriate standard solution to a second 25-mL sample of deionized water.
3. Add 0.2 mL of the same standard to a third 25-mL sample of deionized water.
4. Add 0.3 mL of the same standard solution to a fourth 25-mL sample of deionized water.
5. Analyze each of the solutions according to the appropriate procedure.
6. Tabulate the data as is shown below:

mL standard added	mg/L standard added	mg/L analyte found
0	0	0
0.1	0.2	0.2
0.2	0.4	0.4
0.3	0.6	0.6

These data show several points:

- The standard added to the deionized water was completely recovered. Therefore, the chemicals, instrument, procedure/technique, and standards are working correctly.
- The standard added to the original sample was not completely recovered; therefore, the sample contains an interference.
- Analyses done on the sample will be incorrect. The sample must be pre-treated to remove interferences.

If the results using deionized water give the expected increments (as they did in the example above), go to *Branch C*.

If the results using deionized water do not give the expected increments, the problem may lie with another part of the procedure; go to *Branch D*.

Branch C If interferences are present, the correct results may still be approximated by using multiple standard additions to the sample. Follow the procedure outlined in *Branch B*, substituting sample water for the deionized water.

If the increase in the mg/L of analyte found is uniform for each standard addition increase (ex. 0.1, 0.1, and 0.1), go to *Branch G*.
If the increases are not uniform (i.e., 0.1, 0.08, and 0.05), go to *Branch F*.

Branch D Carefully re-read the instructions for the test.

- Are the correct reagents used in the correct order?
- Is the correct program number selected?
- Is the glassware precise enough?
- Have the reactions been timed properly?

If the procedure technique was incorrect, repeat *Branch B*.
If the procedure technique was correct, go to *Branch E*.

Branch E Check the reagent performance by either obtaining a fresh lot of reagent or by using a known standard to run the test. Make sure the color development time given in the procedure is the same as that required for the reagent in question.

If a reagent is defective, repeat *Branch B* with new reagents.
If the reagents are good, go to *Branch H*.

Branch F Examples **A** and **B** show non-uniform increments between the standard additions produced by the presence of interferences. These data points have been plotted in *Figure 10*.

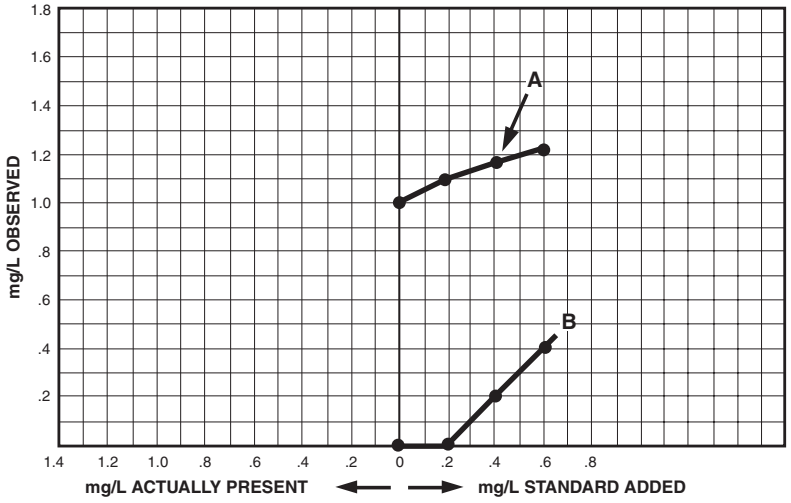
Example A

mL standard added	mg/L standard added	mg/L analyte found
0	0	1.0
0.1	0.2	1.10
0.2	0.4	1.18
0.3	0.6	1.23

Example B

mL standard added	mg/L standard added	mg/L analyte found
0	0	0
0.1	0.2	0
0.2	0.4	0.2
0.3	0.6	0.4

Figure 10 Multiple Standard Additions — A and B



The plot for **Example A** illustrates an interference that becomes progressively worse as the concentration of the standard or analyte increases. This type of interference is uncommon and is more likely to be caused by an error or malfunction of the procedure, reagents, or instrument. Repeat *Branch B* to verify the interference.

The plot for **Example B** illustrates a common chemical interference that becomes less, or even zero, as the concentration of standard or analyte increases. The graph shows the first addition was consumed by the interference and the remaining additions gave the correct increment of 0.2 mg/L. This could also be the result of an error made in the standard addition. Repeat the analysis to see if an error was made during the standard addition. For these two types of interferences, the method is not appropriate for the sample matrix. Analyze the sample by another method that uses a different chemistry.

Branch G Examples **C** and **D** show uniform increments between the standard additions produced by the presence of interferences. These data points have been plotted in *Figure 11*.

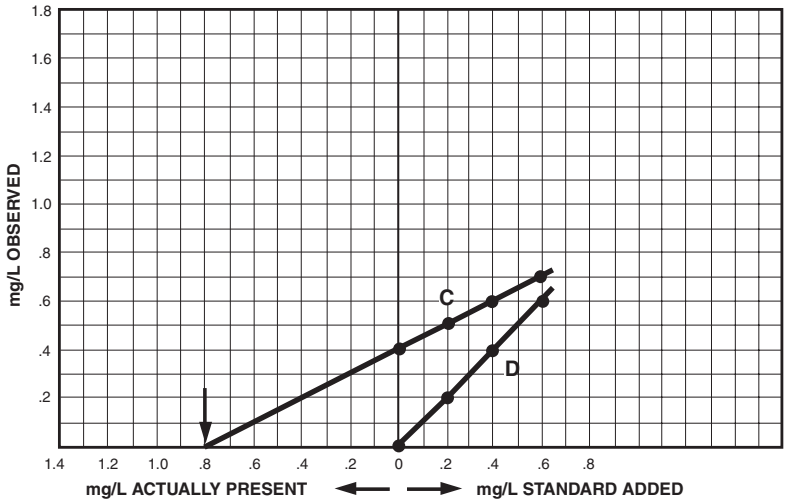
Example C

mL standard added	mg/L standard added	mg/L analyte found
0	0	0.4
0.1	0.2	0.5
0.2	0.4	0.6
0.3	0.6	0.7

Example D

mL standard added	mg/L standard added	mg/L analyte found
0	0	0
0.1	0.2	0.2
0.2	0.4	0.4
0.3	0.6	0.6

Figure 11 Multiple Standard Additions — C and D



The plot for **Example C** illustrates a common interference with a uniform effect on the standard and the substances in the sample. The four data points form a straight line that may be extended back through the horizontal axis. The point where the line meets the axis can be used to determine the concentration of the substance you are measuring.

In this example, the first analysis gave 0.4 mg/L. After extrapolating the line to the horizontal axis, the graph shows the result should be much closer to the correct result: 0.8 mg/L.

The plot for **Example D** illustrates a problem for the analyst. The increments are uniform and the recovery of the standard is complete. The result of the first analysis is 0 mg/L and the extrapolates back through 0 mg/L. If interferences are known to be present, the interferences may be present in an amount equal to the substance in question, preventing the analyst from finding the substance. This would be an uncommon situation.

Apparent interferences may also be caused by a defect in the instrument or standards. Before assuming the interference is chemical, check *Branch B*.

Branch H Check the operation of the instrument and/or apparatus used to perform the test. Return to the main menu. Touch **System Checks** then **Wavelength Check**. Touch **Read**. The wavelength should read 404.66 ± 1 nm. Turn the instrument off then on to perform alignment and wavelength calibration if the wavelength check is out of specification.

Check the glassware used in the procedure and make sure it is extremely clean. Dirty pipettes and graduated cylinders can cause contamination and will not deliver the correct volume.

If a defect is found in the instrument and/or apparatus, repeat *Branch B* after repair or replacement. If the instrument and apparatus are working, proceed with *Branch I*.

Branch I After determining that the procedure, reagents, instrument, and/or apparatus are correct and working properly, the most likely cause for standard additions not functioning correctly in deionized water is the standard used. Obtain a new standard and repeat *Branch B*.

Branch J If the standard additions gives the correct result, the analyst must then determine if one or more interfering substances are present. If interfering substances are present, proceed to *Branch C*. If they are not present, the analysis is correct.

If you cannot identify the problem, call Hach Technical Consultants at 1-800-227-4224 or visit our Web site: http://www.hach.com/forms.htm.

Troubleshooting a Test When Results are in Doubt

If the results from any Hach chemistry are in doubt, troubleshoot them as follows:

1. Run a proof-of-accuracy check. Take a standard solution, which has a known concentration, through the same steps as the original sample. Include sampling and storage, digestion and colorimetric determination, if applicable. If the results of the standard solution check are correct, skip to *step 4* below. If there is a variation in the expected results, go to *step 2*.
2. If the standard solutions check does not match the expected results, check the instrument set-up and method procedure as follows:
   a. Verify that the correct program number for the test being performed is selected.
   b. Verify that the units of concentration of the standard match the displayed units. (One of the alternative forms of the analyte may be in the display.) For example: Molybdenum may be shown as Mo instead of MoO_4.
   c. Verify that the sample cells called for in the procedure are the ones being used.
   d. Verify that the reagents are correct for the sample size being analyzed.
   e. Verify that the reagent blank value stored is for the current procedure. It may be from a previous lot of reagents and therefore not representative of the current reagent lot.

f. Verify the calibration curve adjustment (Standard Adjust) currently in use. The factory-stored default calibration should be used initially to check the standard.

g. Verify that the dilution factor option is correct.

If the instrument setup is correct and the method procedure specifics are being followed correctly, go to *step 3*.

3. If the standard solution check does not match the expected results, check the reagents used in the test and the analytical technique as follows:

a. Determine the age of the reagents used in the test. While most Hach reagents have a long shelf life, many factors affect this (i.e., storage temperature, storage conditions, microbial contamination). Replace suspect reagents and run the standards check again.

b. Run a deionized or distilled water blank through the entire process; include sampling and storage, digestion, and colorimetric determination. Some chemicals will add a small amount of color to a test; this is not considered unusual. However, color development in excess of 10% of the range of the test may indicate a problem with one of the reagents or the dilution water.

c. To troubleshoot the procedure, delete the parts one by one. First, using the standard solution, omit preservation and storage, doing only digestion and colorimetry. If this analysis is correct, examine the procedure used to store the sample. Ensure that it is the procedure prescribed for the chosen parameter. If the sample is acidified for storage, be sure the correct acid is used and the sample is adjusted to the proper pH level before testing.

If the standards check is still incorrect, run the standard on just the colorimetry. If the results are correct, examine the digestion procedure. Ensure that the amount of reagents used and the pH after the digestion are correct for the procedure. (See the procedure for the parameter in question.)

4. If the standard solution gives a correct value, but the results of the sample measurement are questionable, there may be an interference in the sample. To check for an interference:

a. Spike the sample. Use a standard addition test instead of a standard solution test to include any possible interferences.

To a pair of cells containing fresh sample water, add an amount of standard equal to two times the concentration of the sample. Process both samples using the same reagents, instruments, and technique. The spiked sample should show an increase equal to the amount of standard added. Calculate percent recovery as shown below. Ideally, the results should be 100%, with results from 90 to 110% considered acceptable. Refer to the procedure notes for possible interferences and ways to eliminate them.

b. Run a series of dilutions on the sample. Make sure your sample is within the range of the test. A sample out of range for the method may give erroneous results because of under- or over-development of the color, excess turbidity, or even sample bleaching. Run a series of dilutions to check for this possibility.

Because it may not be feasible to determine the cause of the interference, diluting the sample past the point of interference is often the most economical and efficient means of getting the correct result. If it is not possible to dilute out an interference without diluting out the parameter to be measured, use a different method, such as a different chemistry or an ion-selective electrode to measure the parameter.

Calculating percent recovery:

1. Measure the unknown sample concentration.
2. Calculate the theoretical concentration of the spiked sample using the following formula:

$$\text{Theoretical Concentration} = \frac{(C_u \times V_u) + (C_s \times V_s)}{V_u + V_s}$$

Where:

C_u = measured concentration of the unknown sample
V_u = volume of the unknown sample
C_s = concentration of the standard
V_s = volume of the standard

3. Measure the spiked sample concentration.
4. Divide the spiked sample concentration by the theoretical concentration and multiply by 100.

For example:

A sample was tested for manganese and the result was 4.5 mg/L. A separate 97-mL portion of the same sample was spiked with 3 mL of a 100 mg/L standard solution of manganese. This spiked solution was tested again for manganese using the same method. The result was 7.1 mg/L.

The theoretical concentration of the spiked sample is:

$$\frac{(4.5\ \text{mg/L} \times 97\ \text{mL}) + (100\ \text{mg/L} \times 3\ \text{mL})}{97\ \text{mL} + 3\ \text{mL}} = 7.4\ \text{mg/L}$$

The percent spike recovery is:

$$\frac{7.1\ \text{mg/L}}{7.4\ \text{mg/L}} \times 100 = 96\%$$

USEPA Calculation

The USEPA requires a more stringent calculation for percent recovery. This formula calculates the percent recovery only for the standard added to the spiked sample and yields a lower value than the above calculation. A complete explanation for the USEPA formula is in *USEPA Publication SW-846*. The USEPA percent recovery formula is:

$$\%R = \frac{100(X_s - X_u)}{K}$$

Where:

X_s = measured value of the spiked sample
X_u = measured value for the unspiked sample, adjusted for the dilution of the spike volume
K = known value of the spike in the sample

Example:

A sample measures 10 mg/L. A separate 100-mL portion of the sample was spiked with 5 mL of a 100-mg/L standard solution. The spiked solution was measured by the same method as the original sample. The result was 13.7 mg/L.

$$X_s = 13.7 \text{ mg/L}$$

$$X_u = \frac{10 \text{ mg/L} \times 100 \text{ mL}}{105 \text{ mL}} = 9.5 \text{ mg/L}$$

$$K = \frac{5 \text{ mL} \times 100 \text{ mg/L}}{105 \text{ mL}} = 4.8 \text{ mg/L}$$

$$\%R = \frac{100 \times (13.7 \text{ mg/L} - 9.5 \text{ mg/L})}{4.8 \text{ mg/L}} = 88\%$$

Acceptable percent recovery values are 80-120%.

3.2.4 Adjusting the Standard Curve

The DR/2400 Spectrophotometer has over 120 Hach programs permanently installed in memory. A program usually includes a pre-programmed calibration curve. Each curve is the result of an extensive calibration performed under ideal conditions and is normally adequate for most testing. Deviations from the curve can occur from using compromised testing reagents, defective sample cells, incorrect test procedure, incorrect technique, or other correctable causes. Interfering substances or other causes may be beyond the analyst's control.

In some situations, using the pre-programmed curve may not be convenient:

- Running tests where the reagents are highly variable from lot to lot.
- Running tests where frequent calibration curve checks are required.
- Testing samples which give a consistent test interference.

Consider the following before adjusting the calibration curve:

- Will future test results be improved by adjusting the curve?
- Are interfering substances consistent in all the samples that you will test?
- Any estimated detection limit, sensitivity, precision, and test range information provided with the procedure may not apply to an adjusted curve calibration.

The calibration curves can be adjusted by following the steps found in the test procedure. Generally, you add test reagents to a blank and standard solution. Working carefully is important. After the adjustment, it is wise to run standard solutions of several concentrations to make sure the adjusted curve is satisfactory. Performing standard additions on typical samples may also help determine if the adjusted curve is acceptable.

Think of adjusting a measurement as a two-step process. First, the instrument measures the sample using the pre-programmed calibration. Second, it multiplies this measurement by an adjustment factor. The factor is the same for all concentrations The instrument will remember the factor until the program is exited and will display the standard adjustment icon when it is used. You can return to the pre-programmed curve any time by selecting the Hach Program from the main menu.

3.3 Interferences

Interferences are contaminants in a sample that are capable of causing changes in color development, turbidity, or unusual colors and odors, thereby creating errors in your results. A list of common interferences is included in each procedure. Hach reagents are formulated to eliminate many interferences; you can remove others by pretreating the sample as instructed in the procedure.

Test strips are available for many of the common interferences. These can be conveniently used to screen samples for the presence of interferences.

If you get test results that you feel are inaccurate, if you get an unexpected color, or if you notice an unusual odor or turbidity, repeat the test on a sample diluted with deionized water. (See *Section 2.7 Sample Dilution*.) Correct the results for the dilution, and compare them with those from your original test. If they differ significantly, make a second dilution and check it against the first. Repeat the dilutions until you get the same result (after volume corrections) twice in succession.

For more information on interferences, see *Section 3.2.2 Standard Additions*. The *APHA Standard Methods* book, an excellent reference for the water analyst, also covers interferences in its "General Introduction."

pH Interference

Chemical reactions are often pH dependent. Hach reagents contain buffers to adjust the pH of the sample to the correct range. However, the reagent buffer may not be strong enough for samples that are highly buffered or have an extreme pH.

The *Sampling and Storage* section of each procedure gives the pH range for that test. The DR/2400 Spectrophotometer can accept a Platinum Series pH electrode for taking measurements through the instrument.

Before testing, adjust the sample to the proper pH as instructed in the procedure, or by following these steps:

1. Measure the pH of your analyzed sample with a pH meter. Use pH paper when testing for chloride, potassium, or silver.
2. Prepare a reagent blank using deionized water as the sample. Add all reagents called for in the procedure. Timer sequences, etc., may be ignored. Mix well.
3. Measure the pH of the reagent blank with a pH meter.
4. Compare the pH values of your analyzed sample with the reagent blank.
5. If there is little difference in the values of your analyzed sample and the reagent blank, then pH interference is not the problem. Follow the *Accuracy Check* for the specific procedure to more clearly identify the problem.
6. If there is a large difference between the value of your analyzed sample and the reagent blank, adjust the sample pH to the value of the reagent blank. Adjust the sample pH to this same pH for all future samples before analysis. Use the appropriate acid, usually nitric acid, to lower the pH. Use the appropriate base, usually sodium hydroxide, to raise the pH. Adjust the final result for any dilution caused by adding acid or base; see *Correcting for Volume Additions*.
7. Analyze the sample as before.

8. Some purchased standards may be very acidic and will not work directly with Hach procedures. Adjust the pH of these standards as described above. Adjust the final concentration of the standard for the dilution. The Hach standard solutions suggested in the procedures are formulated so that no pH adjustment is necessary.

3.4 Method Performance

3.4.1 Estimated Detection Limit (EDL)

Ranges for chemical measurements have limits. The lower limit is important because it determines whether a measurement is different from zero. Many experts disagree about the definition of this detection limit, and determining it can be difficult. The *Code of Federal Regulations* (40 CFR, Part 136, Appendix B) provides a procedure to determine the "Method Detection Limit" or MDL. The MDL is the lowest concentration that is different from zero with a 99% level of confidence. A measurement below this MDL is highly suspect.

The MDL is not fixed; it varies for each reagent lot, instrument, analyst, sample type, etc. Therefore, a published MDL may be a useful guide, but is only accurate for a specific set of circumstances. Each analyst should determine a more accurate MDL for each specific sample matrix using the same equipment, reagents, and standards that will routinely be used for measurements.

Hach provides a sensitivity value (concentration change equivalent to an absorbance change of 0.010 abs) as an estimate of the lower detection limit of each test. The sensitivity value may be treated as an EDL for the purposes of MDL determination. It can be considered a good starting concentration when determining a MDL. **Do not use the EDL for MDL**. The conditions for MDL determination must be exactly the same as the conditions used for analysis. The EDL may be useful to the analyst as a starting point in determining a MDL, or as a way to compare methods. Measurements below the EDL may also be valuable because they can show a trend, indicate the presence of analyte and/or provide statistical data. However, these values have a large uncertainty.

3.4.2 Method Detection Limit (MDL)

This method is in accordance with the USEPA definition in 40 CFR, Part 136, Appendix B in the 7-1-94 edition. The USEPA defines the method detection limit (MDL) as the minimum concentration that can be determined with a 99% level of confidence that the true concentration is greater than zero. Since the MDL will vary from analyst to analyst, it is important that the MDL be determined under actual operating conditions.

The procedure for determining MDL is based on replicate analyses at a concentration 1 to 5 times the estimated detection limit. The MDL value is calculated from the standard deviation of the replicate study results multiplied by the appropriate Student's *t* value for a 99% confidence interval. For this definition, the MDL does not account for variation in sample composition and can only be achieved under ideal conditions.

1. Estimate the detection limit. Use the Hach sensitivity value stated in the *Method Performance* section of the analysis procedure.
2. Prepare a laboratory standard of the analyte, 1 to 5 times the estimated detection limit, in deionized water that is free of the analyte.

3. Analyze at least seven portions of the laboratory standard and record each result.
4. Calculate the average and the standard deviation(*s*) of the results.
5. Compute the MDL using the appropriate Student's *t* value (see table below) and the standard deviation value:

MDL = Student's *t* x *s*

Number of Test Portions	Student's *t* Value
7	3.143
8	2.998
9	2.896
10	2.821
MDL = Students t × s	

For example:

The EDL for measuring iron using the FerroZine® method is 0.003 mg/L. An analyst accurately prepared 1 liter of a 0.010 mg/L (about 3x the EDL) laboratory standard by diluting a 10-mg/L iron standard in iron-free deionized water.

Eight portions of the standard were tested according to the FerroZine method with the following results:

Sample #	Result (mg/L)
1	0.009
2	0.010
3	0.009
4	0.010
5	0.008
6	0.011
7	0.010
8	0.009

Using a calculator program, the average concentration = 0.010 mg/L and the standard deviation (*s*) = 0.0009 mg/L

Based on the USEPA's definition, calculate the MDL as follows:

MDL for FerroZine method = 2.998 (Student's *t*) x 0.0009 (*s*)

MDL = 0.003 mg/L (agrees with initial estimate)

Note: *Occasionally, the calculated MDL may be very different than Hach's estimate of the detection limit. To test how reasonable the calculated MDL is, repeat the procedure using a standard near the calculated MDL. The average result calculated for the second MDL derivation should agree with the initial calculated MDL. Refer to 40 CFR, Part 136, Appendix B (7-1-94), pages 635–637 for detailed procedures to verify the MDL determination.*

Run a laboratory blank, containing deionized water without analyte, through the test procedure to confirm that the blank measurement is less than the calculated MDL. If the blank measurement is near the calculated MDL, repeat the MDL procedure using a separate blank for analysis for each portion of standard solution analyzed. Subtract the average blank measurement from

each standard and use the corrected standard values to calculate the average and standard deviation used in the MDL.

3.4.3 Precision

Every measurement has some degree of uncertainty. Just as a ruler with markings of 1 mm leaves some doubt as to the exact length of a measurement, chemical measurements also have some degree of uncertainty. The quality of the entire calibration curve determines the precision.

Uncertainty in chemical measurements may be due to systematic errors and/or random errors. A systematic error is a mistake that is always the same for every measurement made. For example, a blank can add to each measurement for a specific compound, giving consistently high results (a positive bias). Random errors are different for every test and can add either a positive or negative variation in response. Random errors are most often caused by variation in analytical technique. Hach chemists work hard to eliminate systematic errors in Hach procedures using Hach reagents, but response variation occurs in all chemical measurements.

3.4.4 Estimating Precision

The method performance section in each procedure provides an estimate of the procedure's precision. Two types of estimates are used throughout thismanual. Most of the procedures use a *replicate analysis* estimate, based on real data. For precision determined in this manner, the 95% confidence interval of the distribution is reported. Some newer procedures use a 95% or 99% *confidence interval*, which is based on the calibration data for that particular chemistry.

In replicate analysis, a Hach chemist prepares a specific concentration of the analyte in a deionized water matrix. The standard is then analyzed seven individual times on a single instrument with the two reagent lots originally used in the calibration (a total of 14 samples). A standard deviation of each of the two sets of seven values is calculated, and the worst-case 95% confidence interval of the distribution is reported in the method. The reported value provides an estimate of the "scatter" of results at a particular point in the calibration curve.

In cases where precision is determined directly from the calibration data, an estimate is obtained from the calibration data itself, with no additional replicate analyses. In this case, the precision is the 95 or 99% confidence interval for the stated concentrations. The precision range is an estimate of the average response variation and is based on multiple reagent lots and instruments used in the calibration. Therefore, it will not exactly predict the true precision range for each reagent lot, but does provide a useful estimate.

In either case, it is important to realize that the estimates are based on a deionized water matrix. Precision on real samples with varying matrices can be quite different from these estimates.

If the concentration obtained from running a standard solution does not fall within the stated precision, please refer to *3.4.3 Precision*. *Troubleshooting a Test When Results are in Doubt*.

3.4.5 Sensitivity

Hach's definition of sensitivity is the change in concentration (ΔConcentration) for a 0.010 change in absorbance (ΔAbs).

Use sensitivity when comparing different methods. For example, Hach has three DR/2400 methods for determining iron:

Iron Analysis Method	Portion of Curve	ΔAbs	ΔConcentration
FerroVer	Entire range	0.010	0.022 mg/L
FerroZine	Entire range	0.010	0.009 mg/L
TPTZ	Entire range	0.010	0.012 mg/L

Notice that the FerroZine method has the greatest sensitivity of the three methods because it will measure the smallest change in concentration. The technical definition of sensitivity comes from a calibration curve with Abs on the x-axis and concentration on the y-axis.

1. If the calibration is a line, the sensitivity is the slope of the line multiplied by 0.010.
2. If the calibration is a curve, the sensitivity is the slope of the tangent line to the curve at the concentration of interest multiplied by 0.010.

The sensitivity value is also used as an estimate of the lower limit of the test. The value may be used as a starting point in determining MDL.

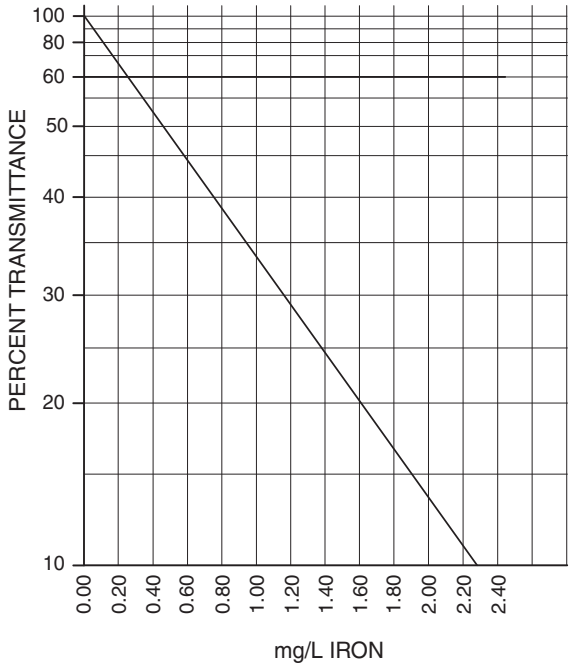
3.5 Preparing a Calibration Curve

1. Prepare five or more standards of known concentration that cover the expected range of the test. Run tests as described in the procedure on each prepared standard. Then pour the customary volume of each known solution into a separate clean sample cell of the type specified for your instrument.
2. Select the proper wavelength. Standardize (zero) the instrument using an untreated water sample or a reagent blank, whichever the procedure instructs you to use.
3. Measure and record the absorbance of the known solutions within the time constraints detailed in the procedure. To use absorbance vs. concentration, see *Section %T Versus Concentration Calibration*.

3.5.1 %T Versus Concentration Calibration

If measuring %T, use semilogarithmic graph paper and plot %T (vertical scale) versus concentration (horizontal scale). For *Figure 12*, iron standard solutions of 0.1, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 mg/L were measured on a Spectronic 20 at 500 nm using half-inch test tubes. Results were plotted and the calibration table values were extrapolated from the curve (*Table 9*).

Figure 12 Semilogarithmic Calibration Curve



To convert %T readings to concentration, prepare a table such as *Table 9* and select the appropriate line from the "%T Tens" column and the appropriate column from the "% T Units" columns. The %T Ten value is the first number of the % transmittance reading and the %T Units value is the second number of the % transmittance reading. For example, if the instrument reading was 46%, the 40 line in the %T Tens column and the 6 column in the %T Units would be selected. The cell where these two intersect (0.78 mg/L) is the iron concentration of the sample.

Table 9 Calibration Table

	Absorbance Units									
Absorbance Tens	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0	–	–	–	–	–	–	–	–	–	–
10	2.30	2.21	2.12	2.04	1.97	1.90	1.83	1.77	1.72	1.66
20	1.61	1.56	1.51	1.47	1.43	1.39	1.35	1.31	1.27	1.24
30	1.20	1.17	1.14	1.11	1.08	1.04	1.02	.99	.97	.94
40	.92	.89	.87	.84	.82.	.80	.78	.76	.73	.71
50	.69	.67	.65	.64	.62	.60	.58	.56	.55	.53
60	.51	.49	.48	.46	.45	.43	.42	.40	.39	.37
70	.36	.34	.33	.32	.30	.29	.28	.26	.25	.24
80	.22	.21	.20	.19	.17	.16	.15	.14	.13	.12
90	.11	.09	.08	.07	.06	.05	.04	.03	.02	.01

3.5.2 Absorbance Versus Concentration Calibration

If absorbance values are measured, plot the results on linear graph paper. Plot the absorbance value on the vertical axis and the concentration on the horizontal axis.

Plot increasing absorbance values from bottom to top. Plot increasing concentration values from left to right. Values of 0.000 absorbance units and 0 concentration will begin at the bottom left corner of the graph. A calibration table can be extrapolated from the curve or the concentration values can be read directly from the graph. Or determine an equation for the line using the slope and y-intercept.

3.6 Adapting Procedures to Other Spectrophotometers

Hach procedures may be used with other spectrophotometers, if calibration curves are made that convert absorbance to concentration. Regardless of the spectrophotometer used, prepare the sample and calibration standards following the Hach procedure and use the optimum wavelength used in the Hach procedure.

To calibrate for a given analyte, a series of standards are prepared and measured to establish the calibration curve. The absorbance vs. concentration is plotted on linear graph paper (as in *Figure 10* and *Figure 11*). Points on the graph are connected with a smooth line (curved or straight). If necessary, use the curve to make a calibration table.

3.6.1 Selecting the Best Wavelength

When developing a new procedure, or using procedures that are sensitive to wavelength, it is normal to select the wavelength where the instrument gives the greatest absorbance (see *Figure 13*). Because Hach chemists have selected the best wavelength for the procedures in this manual, selecting the wavelength is not necessary in most cases.

3.6.1.1 General steps to select the best wavelength on a spectrophotometer:

1. Refer to the instrument manual for specific instructions for wavelength adjustments.
2. Select single wavelength adjustment.
3. Enter a wavelength in the range of interest.
4. Prepare the sample and blank for analysis. Fill the appropriate sample cells with the blank and the sample solutions.
5. Place the blank in the cell holder. Zero the instrument.
6. Place the prepared sample into the cell holder. Read the absorbance level.
7. Increase the wavelength so it is at least 100 nm greater than the range of interest. Re-zero as in step *5.* Measure and record the absorbance of the sample.
8. Repeat, decreasing the wavelength by 50 nm. Re-zero, then measure and record the absorbance at each increment. Continue this process throughout the wavelength range of interest. Note the wavelength of greatest absorbance. (See *Table 10*.)

Note: *Sample color provides a good indication of what wavelength region to use. A yellow solution absorbs light in the 400–500 nm region. A red solution absorbs light between 500–600 nm. A blue solution absorbs light in the 600–700 nm range.*

Table 10

Wavelength	Absorbance
550 nm	0.477
500 nm	0.762
450 nm	0.355
400 nm	0.134

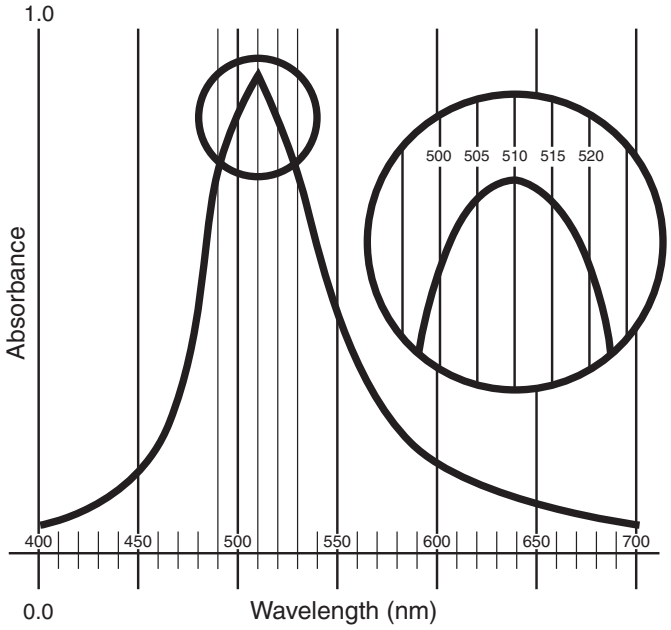
9. Adjust the wavelength to 50 nm more than the highest absorbance point on the initial search (step *8*). Re-zero as in step *5*.
10. Measure and record the absorbance. Repeat, decreasing the absorbance in 5-nm steps. Re-zero, then measure and record the absorbance at each increment. Continue until the entire range of interest is measured (see *Table 11*).

Table 11

Wavelength	Absorbance
520 nm	0.748
515 nm	0.759
510 nm	0.780
505 nm	0.771
500 nm	0.771
495 nm	0.651
490 nm	0.590

Check to be sure there is enough difference in absorbance between samples with low and high analyte concentrations by measuring two sample solutions that contain the expected low and high concentrations of analyte at the optimum wavelength. The change in absorbance caused by increases/decreases in concentration depends on the sensitivity of the procedure and the chemistry. Chemistries with small absorbance changes are less sensitive, but tend to have larger ranges. Chemistries with large absorbance changes are more sensitive, but tend to have smaller ranges.

Figure 13 Selecting the Best Wavelength



Adapting a Buret Titration for Use With a Digital Titrator

Any standard titration procedure that uses a buret can be adapted to the Digital Titrator by using the following procedure.

1. From *Table 12* on page *50*, select a titration cartridge having the same active ingredient as the buret solution.
2. Determine the approximate number of digits required. The Digital Titrator dispenses 1 mL per 800 digits on the counter. Using the following equation, determine the digits required for your buret method.

$$\text{Digits Required} = \frac{N_t \times mL_t \times 800}{N_c}$$

Where:

N_t = Normality of buret titrant

mL_t = milliliters of buret titrant required for an average titration

N_c = Normality of Digital Titrator cartridge.

If the number of digits required is within the range of 70 to 350, you can use the procedure as written, substituting the Digital Titrator directly for the buret.

Or, if the number of digits is outside of this range, make the following modifications:

- **a.** If the number of digits required is greater than 350, decrease the sample size to save titrant.
- **b.** If the number of digits required is less than 70, increase the sample size to increase precision.
- **c.** If the sample size is altered, adjust the amount of buffering or indicating reagents by the same proportion.

3. When using the Digital Titrator for your buret method, note the number of digits required for a sample titration. To convert the digits required to the equivalent number of milliliters for a buret method, calculate:

$$\text{Equivalent Buret Milliliters} = \text{Digits Required} \times \frac{N_c}{800 \times N_t}$$

If the sample size was changed, adjust the equivalent buret milliliters accordingly. If the sample size was increased, reduce the equivalent buret milliliters; if the sample size was reduced, increase the equivalent buret milliliters. Multiply the equivalent buret milliliters by any normally used factors to calculate concentration in oz./gal, g/L, etc.

Example: Adapt a buret procedure that normally requires about 20 mL of a 0.4 N titrant to the Digital Titrator. Try an 8.0 N titration cartridge. The first equation above gives:

$$\text{Digits Required} = \frac{0.4 \times 20 \times 800}{8.0} = 800 \text{ digits}$$

Because this would use excessive titrant, reduce the sample size to one-fourth its normal size to reduce the digits required to 200, well within the recommended range.

Upon completion of the titration using the smaller sample size, calculate the equivalent buret milliliters by the second equation above.

If 205 were the digits required:

$$\text{Equivalent Buret Milliliters} = \frac{205 \times 8.0}{800 \times 0.4} = 5.13 \text{ mL}$$

Multiply the resulting 5.13 mL by four to account for the reduction in sample size and give the true equivalent buret milliliters of 20.5 mL. If the buret method called for multiplying the number of milliliters of titrant by a factor to calculate the concentration of a sample component, then multiply 20.5 by that factor.

Table 12

Description	Cat. No.
Bismuth Nitrate, 0.0200 M	24345-01
CDTA, 0.800 M, HexaVer	14403-01
Ceric Standard Solution, 0.5N	22707-01
EDTA, 0.0800 M, TitraVer	14364-01
EDTA, 0.142 M	14960-01
EDTA, 0.714 M	14959-01
EDTA, 0.800 M, TitraVer	14399-01
FEAS, ferrous ethylenediammonium sulfate, 0.00564 N	22923-01
Hydrochloric Acid, 8.00 N	14390-01
Iodate-Iodide, potassium, 0.3998 N	14961-01
Iodate-Iodide, potassium, 1.00 N	22944-01
Magnesium Chloride, 0.0800 N	20625-01
Mercuric Nitrate, 0.2256 N	14393-01
Mercuric Nitrate, 2.256 N	921-01
Mercuric Nitrate, 2.57 N	23937-01
PAO, phenylarsine oxide, 0.00451 N	22599-01

Table 12 (continued)

Description	Cat. No.
PAO, phenylarsine oxide, 0.0451 N	21420-01
Potassium Dichromate, 1.00 N	21971-01
Silver Nitrate, 0.2256 N	14396-01
Silver Nitrate, 1.128 N	14397-01
Sodium Hydroxide, 0.1600 N	14377-01
Sodium Hydroxide, 0.1612 N	24280-02
Sodium Hydroxide, 0.3636 N	14378-01
Sodium Hydroxide, 0.9274 N	14842-01
Sodium Hydroxide, 1.600 N	14379-01
Sodium Hydroxide, 3.636 N	14380-01
Sodium Hydroxide, 0.9274 N	14842-01
Sodium Hydroxide, 1.600 N	14379-01
Sodium Hydroxide, 3.636 N	14380-01
Sodium Hydroxide, 8.00 N	14381-01
Sodium Thiosulfate, 0.00451 N	24086-01
Sodium Thiosulfate, 0.0451 N	24095-01
Sodium Thiosulfate, 0.02256 N	24091-01
Sodium Thiosulfate, 0.0250 N	24093-01
Sodium Thiosulfate, 0.113 N	22673-01
Sodium Thiosulfate, 0.2000 N	22675-01
Sodium Thiosulfate, 0.2068 N	22676-01
Sodium Thiosulfate, 2.00 N	14401-01
Sodium Vanadate, 0.25 N	22949-01
Sulfuric Acid, 0.1600 N	14388-01
Sulfuric Acid, 1.600 N	14389-01
Sulfuric Acid, 8.00 N	14391-01
TitraVer, 0.0716 M	20817-01
TitraVer, 0.716 M	20818-01

3.7 Comparison of International Drinking Water Guidelines

Table 13 Comparison of International Drinking Water and FDA Bottled Water Guidelines[1]

Parameter	USEPA[2] Maximum Contaminant Level (MCL)	Canada[3] Maximum Acceptable Concentration	EEC[4] Maximum Admissible Concentration	Japan[5] Maximum Admissible Concentration	WHO[6] Guideline	Bottled Water U.S. Federal Drug Administration Level
Aluminum	0.05–0.2 mg/L[7]		0.2 mg/L	0.2 mg/L	0.2 mg/L	
Ammonium			0.5 mg/L	No standard	1.5 mg/L	
Antimony	0.006 mg/L		0.01 mg/L	0.002 mg/L[8]	0.005 mg/L	
Arsenic	0.05 mg/L	0.025 mg/L	0.05 mg/L	0.01 mg/L	0.01 mg/L	0.05 mg/L
Barium	2.0 mg/L	1.0 mg/L	No standard	No standard	0.7 mg/L	2.0 mg/L
Boron		5.0 mg/L	1.0 mg/L	0.2 mg/L[8]	0.3 mg/L	
Cadmium	0.005 mg/L	0.005 mg/L	0.005 mg/L	0.01 mg/L	0.003 mg/L	0.005 mg/L
Chloride	250 mg/L[7]	250 mg/L	250 mg/L	200 mg/L	250 mg/L	
Chromium	0.1 mg/L	0.05 mg/L	0.05 mg/L	0.05 mg/L	0.05 mg/L	0.1 mg/L
Coliforms, total Organisms/100 mL	≤ % positive	0	0 or MPN ≤1	0	0	≤ 1 MF
Coliforms (*E. coli*) Organisms/100 mL	0	0	0	0	0	
Color	15 cu[7]	15 cu	20 mg Pt-Co/L	5 cu	15 cu	<15 cu
Copper	1.3 mg/L[7]	1.0 mg/L	2.0 mg/L	1.0 mg/L	1–2 mg/L	1.0 mg/L
Cyanides	0.2 mg/L	0.2 mg/L	0.05 mg/L	0.01 mg/L	0.07 mg/L	
Fluoride	2.0-4.0 mg/L[7]	1.5 mg/L	0.7-1.5 mg/L	0.8 mg/L	1.5 mg/L	
Hardness			50 mg/L	300 mg/L		
Iron	0.3 mg/L[7]	0.3 mg/L	0.2 mg/L	0.3 mg/L	0.3 mg/L	
Lead	0.015 mg/L	0.01 mg/L	0.01 mg/L	0.05 mg/L	0.01 mg/L	0.005 mg/L
Manganese	0.05 mg/L	0.05 mg/L	0.05 mg/L	0.01–0.05 mg/L	0.1–0.5 mg/L	
Mercury	0.002 mg/L	0.001 mg/L	0.001 mg/L	0.0005 mg/L	0.001 mg/L	0.002 mg/L
Molybdenum				0.07 mg/L	0.07 mg/L	
Nickel	0.1 mg/L		0.02 mg/L	0.01 mg/L[8]	0.02 mg/L	
Nitrate/Nitrite, total	10.0 mg/L as N			10.0 mg/L as N		10 mg/L as N
Nitrates	10.0 mg/L as N	10.0 mg/L as N	50 mg/L	10 mg/L as N	50 mg/L as NO_3^-	
Nitrites	1 mg/L as N	3.2 mg/L	0.1 mg/L	10 mg/L	3 mg/L as NO_2^-	1 mg/L as N
Odor	3 TON[9]		2 dilution no. @ 12 °C; 3 dilution no. @ 25 °C.	3 TON		

Table 13 Comparison of International Drinking Water and FDA Bottled Water Guidelines[1] (continued)

Parameter	USEPA[2] Maximum Contaminant Level (MCL)	Canada[3] Maximum Acceptable Concentration	EEC[4] Maximum Admissible Concentration	Japan[5] Maximum Admissible Concentration	WHO[6] Guideline	Bottled Water U.S. Federal Drug Administration Level
pH	6.5–8.5	6.5–8.5	6.5–9.5	5.8–8.6	6.5–8.5	
Phosphorus			5 mg/L	No Standard		
Phenols		0.002 mg/L	0.5 µg/L C_6H_5OH	0.005 mg/L		
Potassium			12 mg/L	No Standard		
Selenium	0.05 mg/L	0.01 mg/L	0.01 mg/L	0.01 mg/L	0.01 mg/L	0.05 mg/L
Silica Dioxide			10 mg/L	No Standard		
Silver	0.1 mg/L[7]	0.05 mg/L	0.01 mg/L	No standard	No standard	
Solids, total dissolved	500 mg/L[7]	500 mg/L	No standard	500 mg/L	1000 mg/L	
Sodium			75-150 mg/L	200 mg/L	200 mg/L	
Sulfate	250 mg/L[7]	500 mg/L	250 mg/L	No Standard	250 mg/L	
Turbidity	0.5-5 NTU	1 NTU	4 JTU	1–2 units	5 NTU	<5 NTU
Zinc	5 mg/L[7]	5.0 mg/L	No Standard	1.0 mg/L	3.0 mg/L	

[1]To our knowledge, data in this table were accurate and current at the publication date. Contact the regulatory agency in your area for the most current information.

[2]United States Environmental Protection Agency.

[3]These limits are established by Health Canada.

[4]In the EEC (European Economic Community), these limits are set by the European Committee for Environmental Legislation.

[5]In Japan, these limits are established by the Ministry of Health and Welfare.

[6]World Health Organization.

[7]U.S. Secondary MCL.

[8]Identified as a parameter to be regulated in the future.

[9]Threshold Odor Number.

3.7.1 Definitions of USEPA Approved and Accepted

USEPA Approved

The United States Environmental Protection Agency (USEPA) establishes limits for maximum contamination levels of certain constituents in water. It also requires that specific methodology be used to analyze for these constituents. Sometimes the USEPA develops these methods; more often, the USEPA evaluates methods developed by manufacturers, professional groups, and public agencies such as:

- American Public Health Association
- American Water Works Association
- Water Environmental Federation
- American Society for Testing and Materials
- United States Geological Survey
- Association of Official Analytical Chemists

When a method meets the USEPA criteria, it is *approved*. All USEPA approved methods are cited in the *Federal Register* and compiled in the *Code of Federal Regulations*. USEPA-approved methods may be used for reporting results to the USEPA and other regulatory agencies.

USEPA Accepted

Hach has developed several procedures that are equivalent to USEPA approved methods. Even though minor modifications exist, the USEPA has reviewed and accepted certain procedures for reporting purposes. These methods are not published in the *Federal Register,* but are referenced to the equivalent USEPA method in the procedure.

Section 4 Waste Management and Safety

This section provides guidelines for laboratory waste management. These guidelines are only a summary of basic USEPA requirements, and do not relieve the user from complying with the complete regulations contained in the Code of Federal Regulations (CFR). The regulations may change, or additional state and local laws may apply; waste generators are responsible for knowing and following all the laws and regulations that apply to their operations.

4.1 Waste Minimization

Minimizing waste is the most effective way to decrease waste management problems and expense. To do this:

- Use the smallest sample size that will produce accurate results.
- Where possible, choose methods that use reagents that pose fewer hazards.
- Eliminate the need to dispose of out-dated materials by purchasing in smaller quantities.
- Use biodegradable detergents to clean glassware and apparatus unless solvents or acids are specifically required.

4.2 Regulatory Overview

The Resource Conservation and Recovery Act (RCRA) controls all solid waste disposal with an emphasis on hazardous waste. Title 40 Code of Federal Regulations (CFR) part 260 contains the federal hazardous waste disposal regulations issued in accordance with the RCRA. The regulations create a system to identify hazardous wastes and track waste generation, transport, and ultimate disposal from cradle to grave. Each facility involved in hazardous waste management must be registered with the USEPA, with the exception of conditionally exempt small quantity generators.

Federal regulations recognize three categories of generators with those generating larger amounts of waste being under stricter control. The categories are:

Conditionally Exempt Small Quantity Generator — less than 100 kg (220 lb) per month

Small Quantity Generator — between 100 kg (220 lbs) and 1000 kg (2200 lbs) per month

Large Quantity Generator — greater than 1000 kg (2200 lbs) per month.

4.3 Hazardous Waste

4.3.1 Definition

For regulatory purposes, a *hazardous waste* is a material that is subject to special consideration by the USEPA under 40 CFR 261. State or local authorities may also designate additional materials as hazardous waste in their areas.

Many toxic compounds are not regulated, but improper management or disposal may lead to legal problems under CERCLA (Superfund) or common law tort.

The definition given by 40 CFR 261 defines a hazardous waste as a solid waste that is not excluded from regulation and meets one or more of the following criteria:

- It is a discarded commercial chemical product, off-specification species, container residue, or spill residue of materials specifically listed in 40 CFR 261.33 (P- and U-codes);
- It is a waste from a specific source listed in 40 CFR 261.32 (K-code);
- It is a waste from a non-specific source listed in 40 CFR 261.31 (F-code); and/or
- It displays any of the following characteristics of hazardous waste:
  - ignitability
  - corrosivity
  - reactivity
  - toxicity

There are exceptions to these regulations and you should review the regulations to see if you are excluded.

4.3.2 Sample Codes

Hazardous wastes are managed by specific codes assigned in 40 CFR 261.20–261.33. These codes are provided to help you identify hazardous waste. The generator is responsible for making the actual waste code determination.

Selected characteristic waste codes for chemicals which may be generated using Hach methods for water analysis are given in the following table. A complete list of waste codes is found in 40 CFR 261.20 through 40 CFR 261.33.

Characteristic	USEPA Code	Chemical Abstract Services (CAS) No.	Regulatory Level (mg/L)
Corrosivity	D002	na	na
Ignitability	D001	na	na
Reactivity	D003	na	na
Arsenic	D004	6440-38-2	5.0
Barium	D005	6440-39-3	100.0
Benzene	D018	71-43-2	0.5
Cadmium	D006	7440-43-9	1.0
Chloroform	D022	67-66-3	6.0
Chromium	D007	7440-47-3	5.0
Lead	D008	7439-92-1	5.0
Mercury	D009	7439-97-6	0.2
Selenium	D010	7782-49-2	1.0
Silver	D011	7440-22-4	5.0

4.3.3 How to Determine if Waste is Hazardous

Federal laws do not require you to test a material to decide if it is a hazardous waste. If the product is not specifically listed in the regulations, you must apply product or generator knowledge to decide if it is hazardous. Often, there is enough information on a Material Safety Data Sheet (MSDS) to decide. Look for characteristics of a hazardous waste:

- Flash point is below 60 °C (140 °F), or it is classified by DOT as an oxidizer (D001).
- The pH of the material is ≤2 or ≥12.5 (D002).
- The material is unstable, reacts violently with water, may generate toxic gases when mixed with water (D003).
- It is toxic (D004–D043).

Use the chemical composition data to decide if a material is toxic based on the concentration of certain contaminants (Heavy metals and a number of organic compounds). If the waste is a liquid, compare the concentration of contaminants to the concentrations listed in 40 CFR 26. If the waste is a solid, analyze the sample by the Toxicity Characteristic Leachability Procedure (TCLP) and then compare the results to the concentrations in 40 CFR 261.24. Levels above the threshold amounts are considered hazardous.

For more information on using the MSDS, see *Material Safety Data Sheets on page 60*.

Some Hach tests use or produce a number of chemicals that make the end product a hazardous waste; for example, the COD tests and Nessler's reagent. Hazardous waste status may also result from substances present in the sample.

4.3.4 Disposal

Hazardous waste must be managed and disposed of according to federal, state, and local regulations. The waste generator is responsible for making hazardous waste determinations. Analysts should check with their facility's environmental compliance department for specific instructions.

Most hazardous wastes should be handled by treatment, storage, and disposal facilities (TSDF) that have USEPA permits. In some cases, the generator may treat the hazardous waste, but may need a permit from the USEPA and/or state agency. Laboratories are not exempt from these regulations. If your facility is a "Conditionally Exempt Small Quantity Generator," special rules may apply. Check 40 CFR 261 to determine if you have to comply with all the laws.

The most common allowed treatment is elementary neutralization. This applies to wastes that are hazardous only because they are corrosive, or are listed only for that reason. Neutralize acidic solutions by adding a base such as sodium hydroxide; neutralize basic solutions by adding an acid such as hydrochloric acid. Slowly add the neutralizing agent while stirring. Monitor the pH. When it is at or near 7, the material is neutralized and may be flushed down the drain. Many wastes generated from Hach procedures may be treated in this manner.

Other chemical or physical treatments such as cyanide destruction or evaporation may require a permit. Check with your environmental department or local regulators to determine which rules apply to your facility.

Laboratory chemicals may be mixed and disposed of with other hazardous wastes generated at your facility. They may also be accumulated in accordance with 40 CFR 262.34 satellite accumulation rules. After collection they may be disposed of in a *labpack*. Many environmental and hazardous waste companies offer labpacking services. They will inventory, sort, pack, and arrange for proper disposal of hazardous waste. Find companies offering these services in the Yellow Pages under "Waste Disposal — Hazardous" or contact state and local regulators for assistance.

4.4 Management of Specific Wastes

Hach has several documents to assist customers in managing waste that has been generated from our products. You can obtain the following documents by calling 1-800-227-4224 or 970-669-3050 and requesting the literature codes given:

Literature Code	Title
9323	Mercury Waste Disposal Firms
9324	RCRA Waste Disposal Information
9325	COD Waste Disposal Information
9326	COD Heavy Metal Concentrations

4.4.0.1 Special Considerations for Cyanide-Containing Materials

Several procedures in this manual use reagents that contain cyanide compounds. These materials are regulated as reactive waste (D003) by the Federal RCRA. Instructions provided with each procedure explain how to collect these materials for proper disposal. It is imperative that these materials be handled safely to prevent the release of hydrogen cyanide gas (an extremely toxic material with the smell of bitter almonds). Most cyanide compounds are stable, and can be safely stored for disposal, in highly alkaline solutions (pH >11) such as 2 N sodium hydroxide. Never mix these wastes with other laboratory wastes that may contain lower pH materials such as acids or even water.

If a cyanide-containing compound is spilled, avoid exposure to hydrogen cyanide gas. Take the following steps to destroy the cyanide compounds in an emergency:

1. Use a fume hood, supplied air, or self-contained breathing apparatus.
2. While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and either calcium hypochlorite or sodium hypochlorite (household bleach).
3. Add an excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.
4. Neutralize the solution and flush it down the drain with a large amount of water. If the solution contains other regulated materials such as chloroform or heavy metals, it may still need to be collected for hazardous waste disposal. Never flush untreated hazardous wastes down the drain.

4.5 Resources

Many sources of information on proper waste management are available. The USEPA has a hotline number for questions about the Resource Conservation and Recovery Act (RCRA). The RCRA Hotline number is 1-800-424-9346. You may also get a copy of the appropriate regulations. Federal hazardous waste regulations are found in 40 CFR 260-99. Obtain this book from the U.S. Government Printing Office or an alternate vendor. Other documents that may be helpful to the hazardous waste manager in the laboratory include:

1. Task Force on Laboratory Waste Management. *Laboratory Waste Management, A Guidebook*; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1994.
2. Task Force on Laboratory Waste Management. *Waste Management Manual for Laboratory Personnel*; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1990.

3. Task Force on Laboratory Waste Management. *Less is Better*; 2nd ed.; American Chemical Society, Department of Government Relations and Science Policy: Washington, DC 1993.
4. Committee on Chemical Safety. *Safety in Academic Chemistry Laboratories*, 5th ed.; American Chemical Society: Washington, DC, 1990.
5. Armour, Margaret-Ann. *Hazardous Laboratory Chemicals Disposal Guide*; CRC Press: Boca Raton, FL, 1991.
6. *Environmental Health and Safety Manager's Handbook*; Government Institutes, Inc.: Rockville, MD, 1988.
7. Lunn, G.; Sansone, E.B. *Destruction of Hazardous Chemicals in the Laboratory*; John Wiley and Sons: New York, 1990.
8. National Research Council. *Prudent Practices for Disposal of Chemicals from Laboratories*; National Academy Press: Washington, DC, 1983.
9. National Research Council. *Prudent Practices for Handling Hazardous Chemicals in Laboratories*; National Academy Press: Washington, DC, 1981.
10. Environmental Protection Agency, Office of Solid Waste and Emergency Response. *The RCRA Orientation Manual*; U.S. Government Printing Office: Washington, DC, 1991.
11. Environmental Protection Agency, Office of Solid Waste and Emergency Response. *Understanding the Small Quantity Generator Hazardous Waste Rules: A Handbook for Small Business*; U.S. Government Printing Office: Washington, DC, 1986.

4.6 Safety

Safety is the responsibility of every analyst. Many of the procedures in this manual use potentially hazardous chemicals and equipment; it is important to prevent accidents by practicing good laboratory techniques. The following guidelines apply to water analysis and are not intended to cover every aspect of safety.

4.6.1 Read Labels Carefully

Read each reagent label carefully. Pay particular attention to the precautions given. Never remove or cover the label on a container while it contains reagent. Do not put a different reagent into a labeled container without changing the label. When preparing a reagent or standard solution, label the container clearly. If a label is hard to read, re-label promptly according to your facility's hazard communication program.

Warning labels also appear on some of the apparatus used with the test procedures. The protective shields with the COD Reactor and the Digesdahl Digestion Apparatus point out potential hazards. Be sure these shields are in place during use and observe the precautions on the label.

4.6.2 Protective Equipment

Use the right protective equipment for the chemicals and procedures. The MSDS contains this information. Protective equipment may include:

- Eye protection, such as safety glasses or goggles, to protect from flying objects or chemical splashes.

- Gloves to protect skin from toxic or corrosive materials, sharp objects, very hot or very cold materials, or broken glass. Use tongs or finger cots when transferring hot apparatus.
- Laboratory coats or splash aprons to protect skin and clothing from splashes.
- Footwear to protect feet from spills. Open toed shoes should not be worn in chemistry settings.
- Respirators may be needed if adequate ventilation, such as fume hoods, are not available. Use fume hoods when directed to do so by the procedure, or as recommended in the MSDS. For many procedures, adequate ventilation is enough if there is plenty of fresh air and air exhaust to protect against unnecessary exposure to chemicals.

4.6.3 First Aid Equipment and Supplies

Most first aid instructions for chemical splashes in eyes or on skin call for thorough flushing with water. Laboratories should have eyewash and shower stations. For field work, carry a portable eyewash unit. Laboratories should also have appropriate fire extinguishers and fume hoods.

4.6.4 General Safety Rules

Follow these rules when working with toxic and hazardous chemicals:

1. **Never** pipet by mouth. Always use a mechanical pipet or pipet bulb to avoid ingesting chemicals.
2. Follow test procedures carefully and observe all precautionary measures. Read the entire procedure before beginning.
3. Wipe up all spills promptly. Get proper training and have the right response equipment to clean up spills. See your safety director for more information.
4. **Do not** smoke, eat, or drink in an area where toxic or irritating chemicals are used.
5. Use reagents and equipment only as directed in the test procedure.
6. **Do not** use damaged labware and broken equipment.
7. Minimize all chemical exposures. **Do not** breathe vapors or let chemicals touch your skin. Wash your hands after using chemicals.
8. Keep work areas **neat** and **clean**.
9. **Do not** block exits or access to emergency equipment.

4.7 Material Safety Data Sheets

Material safety data sheets (MSDS) describe the hazards of chemical products. This section explains the information found on a Hach MSDS, and tells how to locate important information for safety and waste disposal. The information provided on the MSDS applies to the product as sold by Hach. The properties of any mixtures obtained by using this product will be different.

4.7.1 How to Obtain an MSDS

Hach ships a MSDS to each customer with the first order of any chemical product. A new MSDS may be sent when the information on the data sheet is updated. Please review all new MSDS for new information. If you need another copy of an MSDS, simply call 1-800-227-4224 or download directly from www.hach.com/msdsinfo.htm.

4.7.2 Sections of an MSDS

Each MSDS has ten sections. The sections and the information found in them are described below.

Header Information

The Hach catalog number, MSDS date, change number, company address and telephone number, and emergency telephone numbers are listed at the top of the MSDS.

4.7.2.1 Product Identification

This section contains:

- Hach product name
- Chemical Abstract Services (CAS) number
- Chemical name
- Chemical formula, if appropriate
- Chemical family to which the material belongs

4.7.2.2 Ingredients

This section lists each component in the product. It contains the following information for each component:·

- **PCT:** Percent by weight of this component
- **CAS NO.:** Chemical Abstract Services (CAS) registry number for this component
- **SARA:** Superfund Amendments and Reauthorization Act, better known as the "Community Right to Know Law", informs if the component is listed in SARA 313. If the component is listed and you use more than the specified amount, you must report this to the USEPA every year.
- **TLV:** Threshold Limit Value. The maximum airborne concentration for an 8-hour exposure that is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).
- **PEL:** Permissible Exposure Limit. The maximum airborne concentration for an 8-hour exposure that is regulated by the Occupational Safety and Health Administration (OSHA).
- **HAZARD:** Physical and health hazards of the component are explained.

4.7.2.3 Physical Data

The physical properties of the product are given in this section. They include the physical state, color, odor, solubility, boiling point, melting point, specific gravity, pH, vapor density, evaporation rate, corrosivity, stability, and storage precautions.

4.7.2.4 Fire, Explosion Hazard And Reactivity Data

This section contains the flash point and flammable limits of the material. It also includes how to fight fires if the material catches on fire. Key terms in this section include:

- Flash point: The temperature at which a liquid will give off enough flammable vapor to ignite.
- Flammability and ignitability are usually defined by the flash point.

- Lower Flammable Limit (LFL or LEL): The lowest concentration that will produce a fire or flash when an ignition source is present.
- Upper Flammable Limit (UFL or UEL): The vapor concentration in air above which the concentration is too rich to burn.
- NFPA Codes: The National Fire Protection Association (NFPA) has a system to rate the degree of hazards presented by a chemical. These codes are usually placed in a colored diamond. The codes range from 0 for minimal hazard to 4 for extreme hazard. They are grouped into the following hazards: health (blue), flammability (red), reactivity (yellow), and special hazards (white).

4.7.2.5 Health Hazard Data

This section describes the pathways by which the chemical can enter your body (i.e., ingestion, inhalation, skin contact). It also gives acute (immediate) and chronic (long-term) health effects. If the material causes cancer or genetic damage, it is stated in this section.

4.7.2.6 Precautionary Measures

This section contains special precautions for the material. These may include special storage instructions, handling instructions, conditions to avoid, and protective equipment required to use this material safely.

4.7.2.7 First Aid

First aid instructions for exposures to the chemical are given in this section. Be sure to read this section before inducing vomiting in a victim. Some chemicals are better treated by not inducing vomiting. Seek prompt medical attention for all chemical exposures.

4.7.2.8 Spill And Disposal Procedures

This section explains safe practices for cleaning up and disposing of spilled material. For more information, see *Section 4.3 Hazardous Waste* on page *55*. **The waste generator is ultimately responsible for meeting the federal, state, and local laws that apply to his facility.**

4.7.2.9 Transportation Data

Domestic and International shipping information is provided in this section. It gives shipping name, hazard class, and ID number of the product.

4.7.2.10 References

This section lists the reference materials used to write the MSDS.

Following the Reference section, the product will be listed as having SARA 313 chemicals or California Proposition 65 List Chemicals, if applicable. Also found here is any special information about the product.

4.7.3 OSHA Chemical Hygiene Plan

The Occupational Safety and Health Administration (OSHA) enforces laws controlling exposure to hazardous chemicals in laboratories. These regulations are in Title 29 CFR 1910.1450. The regulations apply to all employers who use hazardous chemicals, and require employers to develop and use a written Chemical Hygiene Plan and to appoint a qualified person as the Chemical Hygiene Officer.

Method Identification Number

Approval of Acceptance of method by United States EPA where applicable

Procedure Name

Types of samples analyzed

Name of method used

Reference for method used

Range with units of measure

Reference for USEPA approval or acceptance

Additional Information that may be applicable

Instrument display

Procedure step

Keystrokes required

Illustration of procedure steps

HACH® DR/2400

Sulfide

★Method 8131

Methylene Blue Method*

(5 to 800 µg/L)

Scope and Application: For testing total sulfides, H_2S, HS^-, and certain metal sulfides in groundwater, wastewater brines, and seawater; USEPA Approved for reporting wastewater analysis**

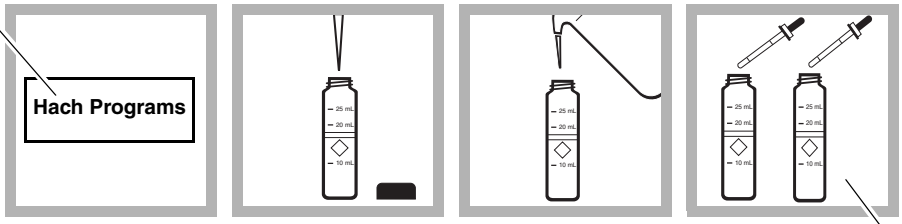
* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

** Procedure is equivalent to USEPA method 376.2 and Standard Method 4500-S^{2-} D for wastewater.

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- Avoid excessive agitation of samples to minimize sulfide loss.
- Some sulfide loss may occur if dilution is necessary.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Sulfide 2 reagent contains potassium dichromate. The final solution will contain hexavalent chromium (D007) at a concentration regulated as a hazardous waste by Federal RCRA. Please see *Section 4* for further information on proper disposal of these materials.

Method 8131

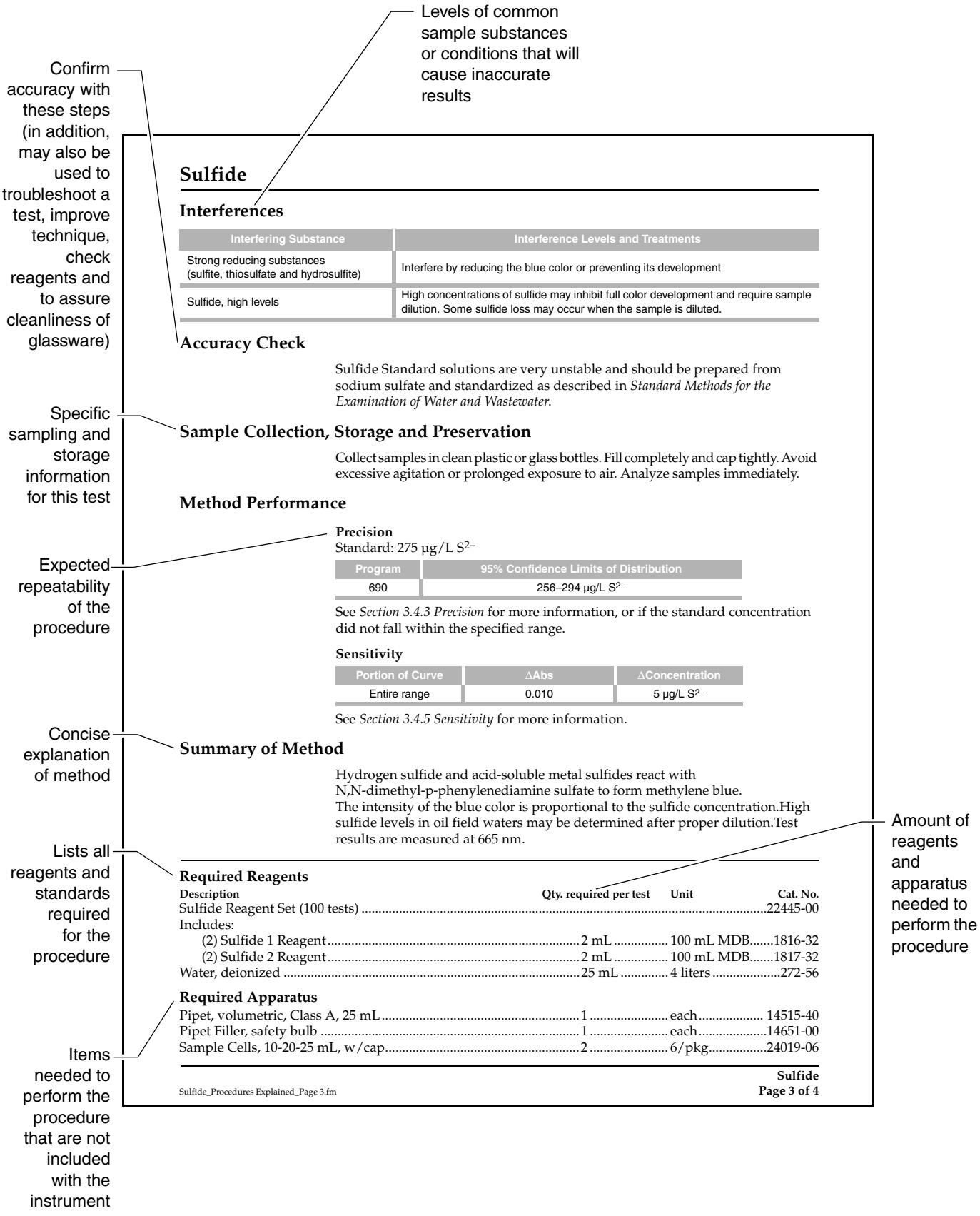


1. Touch **Hach Programs**. Select program **690 Sulfide**. Touch **OK**.

2. Avoiding excess agitation of the sample, use a pipet to add 25 mL of sample to a sample cell (the prepared sample).

3. Measure 25 mL of deionized water into a second sample cell (the blank).

4. Using the calibrated 1-mL dropper, add 1.0 mL of Sulfide 1 Reagent to each cell. Swirl to mix.



Sulfide

Interferences

Interfering Substance	Interference Levels and Treatments
Strong reducing substances (sulfite, thiosulfate and hydrosulfite)	Interfere by reducing the blue color or preventing its development
Sulfide, high levels	High concentrations of sulfide may inhibit full color development and require sample dilution. Some sulfide loss may occur when the sample is diluted.

Accuracy Check

Sulfide Standard solutions are very unstable and should be prepared from sodium sulfate and standardized as described in *Standard Methods for the Examination of Water and Wastewater.*

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation or prolonged exposure to air. Analyze samples immediately.

Method Performance

Precision
Standard: 275 µg/L S^{2-}

Program	95% Confidence Limits of Distribution
690	256–294 µg/L S^{2-}

See *Section 3.4.3 Precision* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	5 µg/L S^{2-}

See *Section 3.4.5 Sensitivity* for more information.

Summary of Method

Hydrogen sulfide and acid-soluble metal sulfides react with N,N-dimethyl-p-phenylenediamine sulfate to form methylene blue. The intensity of the blue color is proportional to the sulfide concentration.High sulfide levels in oil field waters may be determined after proper dilution.Test results are measured at 665 nm.

Required Reagents

Description	Qty. required per test	Unit	Cat. No.
Sulfide Reagent Set (100 tests)			22445-00
Includes:			
(2) Sulfide 1 Reagent	2 mL	100 mL MDB	1816-32
(2) Sulfide 2 Reagent	2 mL	100 mL MDB	1817-32
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Pipet, volumetric, Class A, 25 mL	1	each	14515-40
Pipet Filler, safety bulb	1	each	14651-00
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Sulfide_Procedures Explained_Page 3.fm

Sulfide
Page 3 of 4

Alachlor

Immunoassay Method*

Scope and Application: For water

* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

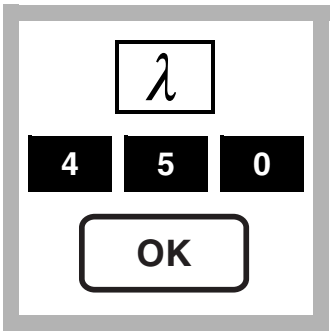
This method analyzes for Alachlor in water. Sample calibrators and reagents are added to cuvettes coated with Alachlor-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

Tips and Techniques

- **Read the entire procedure before starting.** Identify and make ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis. A 1-cm square cell holder is required for this procedure.
- **Timing is critical**; follow instructions carefully.
- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack on page 5.* Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the DR/2400 Spectrophotometer.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.

Note: *Hach Company recommends wearing protective nitrile gloves for this procedure.*

Immunoassay Procedure for Water



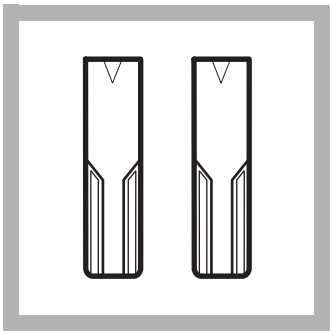
1. Touch

Single Wavelength

then touch the λ button. Type in

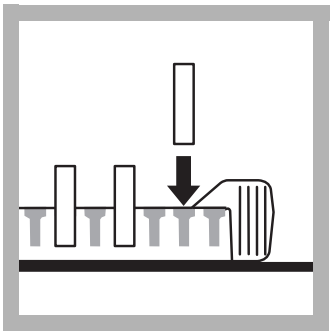
450 nm

and touch **OK.**

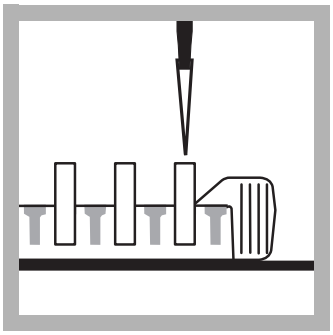


2. Label an Antibody Cuvette for each calibrator and each sample to be tested.

Note: *As many as 20 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.*

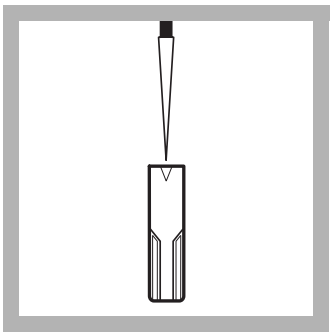


3. Place the cuvettes into the rack snugly.



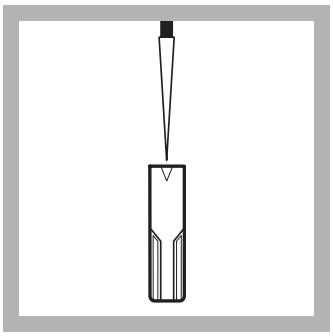
4. Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

Note: *Use a new pipette tip for each calibrator.*

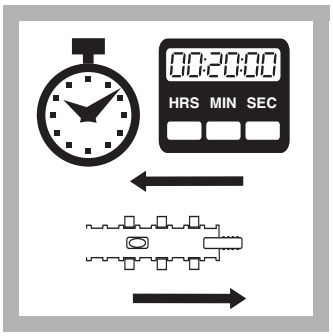


5. Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

Note: *Use a new pipette tip for each sample.*



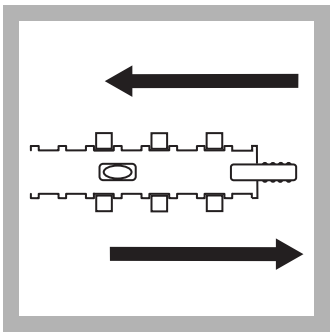
6. Immediately pipet 0.5 mL of Alachlor Enzyme Conjugate into each cuvette.



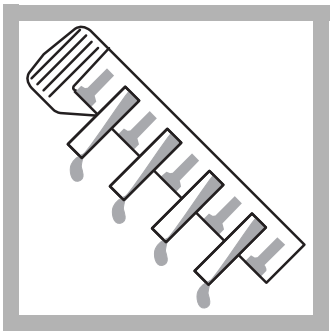
7. Touch the timer icon. Enter 20 minutes and touch **OK.**

A 20-minute reaction time will begin.

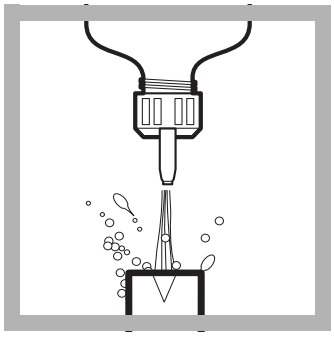
Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack on page 5*.



8. After 10 minutes mix the contents of the rack for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack on page 5*.



9. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

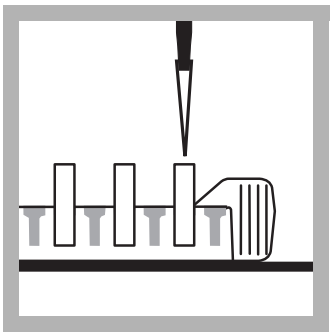


10. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: *Ensure that most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.*

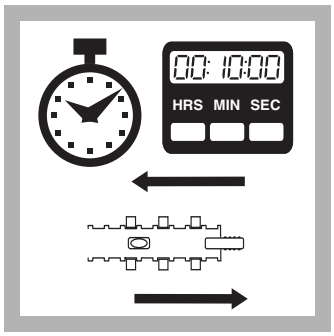
Color Development

Note: *Timing is critical; follow instructions carefully.*



11. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: *Use a new pipette tip for each cuvette.*

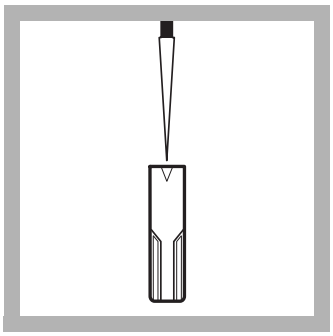


12. Touch the timer icon. Enter 10 minutes and touch **OK.**

A reaction period will begin. Mix, using the instructions on page 5.

13. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Note: *Solutions will turn blue in some or all of the cuvettes.*



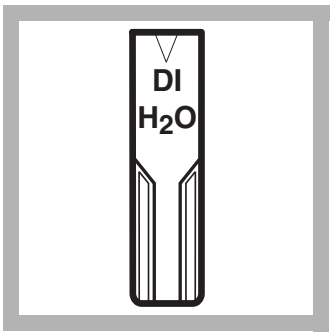
14. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in *step 11*.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

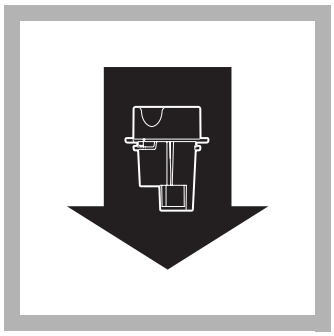
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

Note: *The same pipette tip can be used repeatedly for this step.*

Measuring the Color



15. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

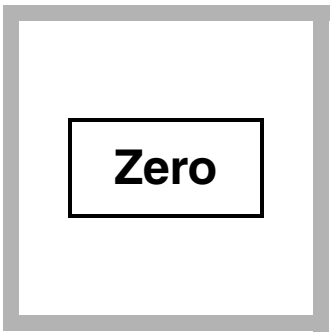


16. Install the 1-cm square cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Place the filled zeroing cuvette into the cell holder—arrow pointing towards the left side of the instrument.

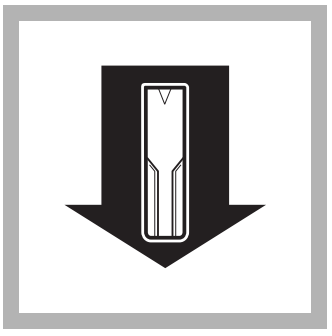
Orient the arrow in the same direction for all cuvettes.



17. Touch **Zero**.

The display will show:

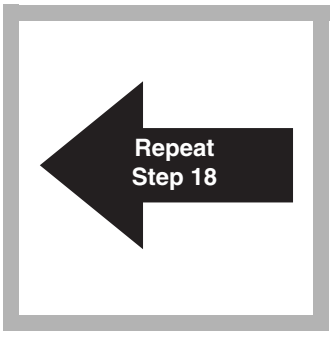
0.000 Abs



18. Place the first calibrator into the cell adapter.

Touch **Read**.

The display will give an absorbance reading. Record the results for each calibrator and sample.



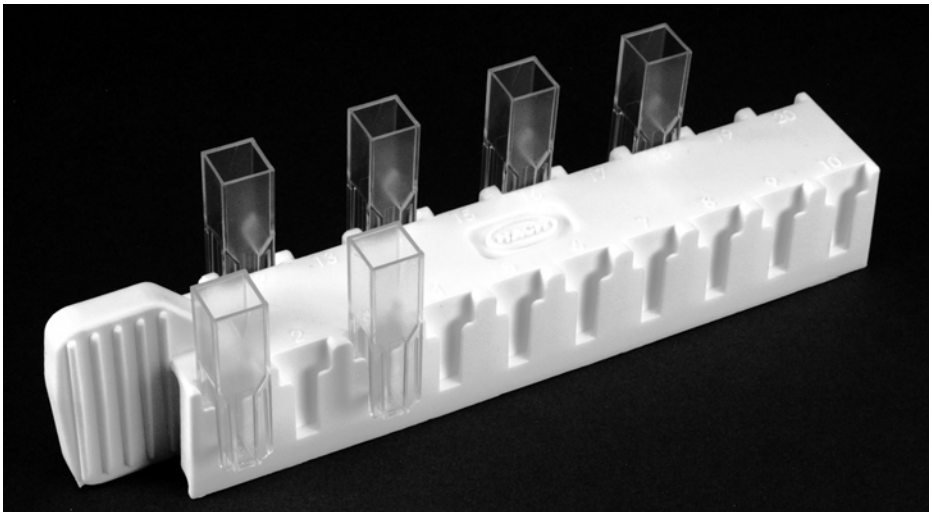
19. Repeat *step 18* for all remaining calibrators and samples.

See *Interpreting and Reporting Results* for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 14*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 14 The 1-cm MicroCuvette Rack



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of Alachlor and the reading. In other words, the higher the reading, the lower the concentration of Alachlor.

If the sample reading is...	the sample Alachlor Concentration is...
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example

Readings:

0.1 ppb Alachlor Calibrator: **0.475 Abs**

0.5 ppb Alachlor Calibrator: **0.245 Abs**

Sample #1: **0.140 Abs**

Sample #2: **0.300 Abs**

Sample #3: **0.550 Abs**

Interpretation

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Alachlor is greater than both 0.1 ppb and 0.5 ppb Alachlor.

Sample #2 — Sample reading is between the readings for the 0.1 ppb and 0.5 ppb Alachlor calibrators. Therefore the sample concentration of Alachlor is between 0.1 ppb and 0.5 ppb.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Alachlor is less than both 0.5 ppb and 0.1 ppb.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Alachlor immunoassay test cannot differentiate between certain herbicides and metabolites, but it detects their presence to differing degrees. The following table shows the required concentration for selected chemicals.

Compound	Concentration to give a positive response of 0.1 ppb Alachlor	Concentration to give a positive response of 0.5 ppb Alachlor
Acetochlor	0.45 ppb	4 ppb
Butachlor	0.09 ppm	1 ppm
2 Chloro-2',6'-Diethylacetaniline	0.030 ppm	2 ppm
Metolachlor	0.085 ppm	2 ppm
2,6-Diethylaniline	0.3 ppm	9 ppm
Propachlor	0.72 ppb	12 ppb

Sample Collection and Storage

The following compounds are not detectable at 10,000 ppb:		
Atrazine	Carbofuran	Carbendazim
Aldicarb	2, 4-D	
Diazoton	Chlorpyirfos	

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Diluting Water Samples

Other levels of Alachlor can be tested by diluting the sample and comparing the results to the 0.1 ppb Calibrator. Select the appropriate sample volume from the table below, place it in a graduated mixing cylinder, and dilute it to 50 mL with deionized water.

mL Sample	Representative Concentration using 0.1 ppb Calibrator
0.5	10 ppb
1.0	5 ppb
2.5	2 ppb
5.0	1 ppb

Example:

Dilute 2.5 mL of sample to 50 mL with deionized water. Run the diluted sample in the procedure along with the 0.1 ppb calibrator. If the absorbance of the diluted sample is less than the 0.1 ppb calibrator, the concentration of the original sample is greater than 2 ppb.

Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Alachlor-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Alachlor from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Alachlor compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Alachlor and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Alachlor in the sample. The resulting color is then compared with a calibrator to determine whether the Alachlor concentration in the sample is greater or less than the threshold levels. Test results are measured at 450 nm.

Alachlor

Required Reagents

Description	Unit	Cat. No.
Reagent Set, Alachlor*	20 cuvettes	28130-00

Required Apparatus

Adapter, 1-cm square cell	each	59459-00
Caps, flip spout	2/pkg	25818-02
Cell holder, 1-cm square	each	59065-00
Marker, laboratory	each	20920-00
Rack, for 1-cm Micro Cuvettes	each	48799-00
Wipes, disposable	box	20970-00
TenSette®, Pipet, 0.1–1.0 mL	each	19700-01
Tips, for pipettor 19700-01	1000/pkg	21856-28

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.

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On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Aluminum

Method 8012

Aluminon Method*

Powder Pillows

(0.008 to 0.800 mg/L)

Scope and Application: For water and wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- Digestion is required for determining total aluminum. See *Section 4* on page *55* for the digestion procedure.
- The sample temperature must be between 20–25 °C (68–77 °F) for accurate results.
- Clean all glassware with 6.0 N HCl and deionized water before use to remove contaminants from the glass.
- After adding the AluVer® 3 Aluminum Reagent Powder Pillow, an orange to orange-red color will develop if aluminum is present.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Clean the graduated cylinder and sample cells with soap and a brush immediately following the test.

Powder Pillows — Method 8012

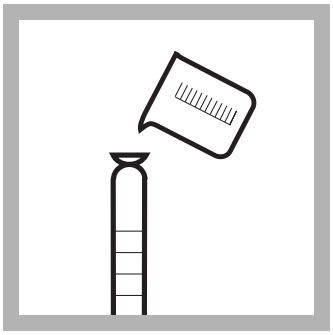
1. Touch

Hach Programs.

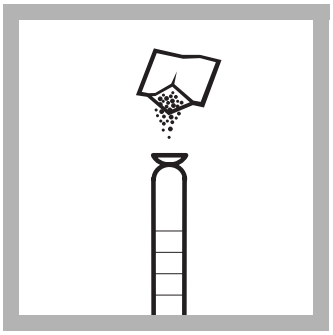
Select program

10 Aluminum, Alumin.

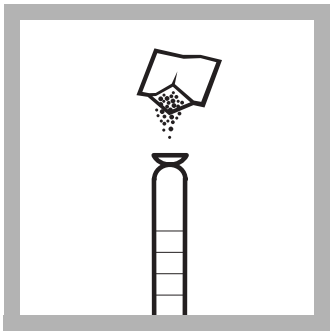
Touch **Start.**



2. Fill the cylinder to the 50-mL mark with sample.

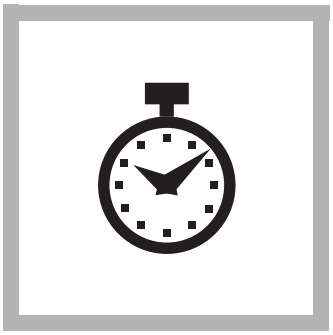


3. Add the contents of one Ascorbic Acid Powder Pillow. Stopper. Invert several times to dissolve powder.



4. Add the contents of one AluVer 3 Aluminum Reagent Powder Pillow.

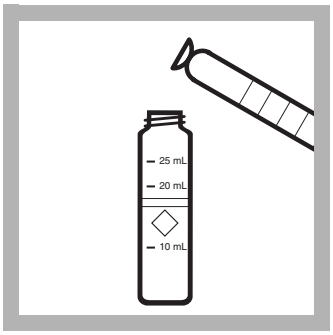
Stopper.



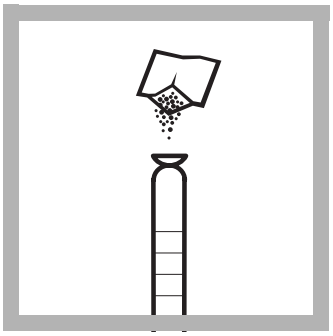
5. Touch the timer icon.

Touch **OK**.

Invert repeatedly for one minute to dissolve the powder. Undissolved powder will cause inconsistent results.

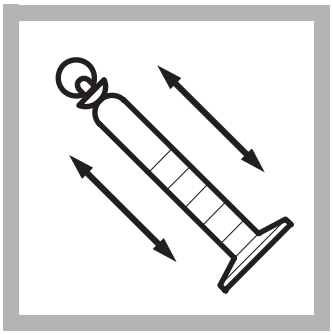


6. Pour 25 mL of the mixture into a 25-mL sample cell. (This is the prepared sample.)



7. Add the contents of one Bleaching 3 Reagent Powder Pillow to the remaining 25 mL in the graduated cylinder.

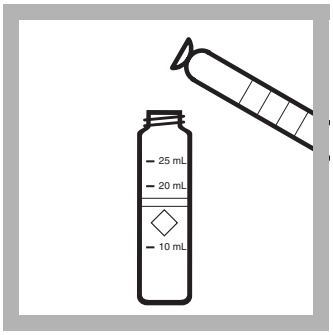
Stopper.



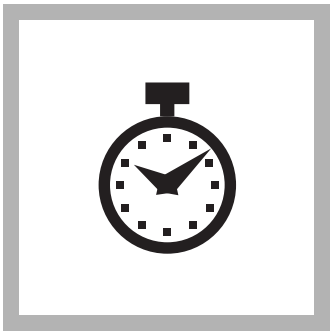
8. Touch the timer icon.

Touch **OK**.

Vigorously shake for 30 seconds. (This solution should turn a light to medium orange.)



9. Pour the 25 mL of solution from the cylinder into a second 25-mL sample cell. (This is the blank.)

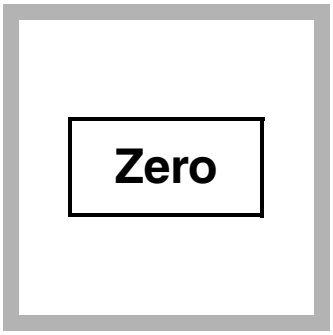


10. Touch the timer icon.

Touch **OK**.

A 15-minute reaction period will begin.

11. Within 5 minutes of the timer beep, wipe the blank and place it into the cell holder.

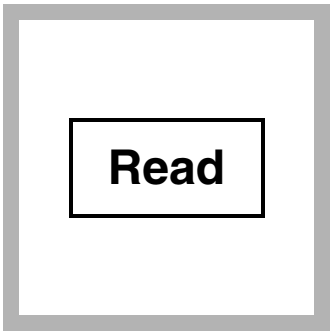


12. Touch **Zero**.

The display will show:

0.000 mg/L Al^{3+}

13. Immediately wipe the prepared sample and place it into the cell holder.



14. Touch **Read**.

Results will appear in mg/L Al^{3+}.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 300 mg/L as $CaCO_3$. Samples with greater than 300 mg/L acidity as $CaCO_3$ must be treated as follows: a) Add one drop of m-Nitrophenol Indicator Solution (Cat. No. 2476-32) to the sample taken in *step 3*. b) Add one drop of 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-26). Stopper the cylinder. Invert to mix. Repeat as often as necessary until the color changes from colorless to yellow. c) Add one drop of 5.25 N Sulfuric Acid Standard Solution (Cat. No. 2449-32) to change the solution from yellow back to colorless. Continue with the test.
Alkalinity	1000 mg/L as $CaCO_3$. Interferences from higher alkalinity concentrations can be eliminated by the following pretreatment: a) Add one drop of m-Nitrophenol Indicator Solution (Cat. No. 2476-32) to the sample taken in *step 2*. A yellow color indicates excessive alkalinity. b) Add one drop of 5.25 N Sulfuric Acid Standard Solution (Cat. No. 2449-32). Stopper the cylinder. Invert to mix. If the yellow color persists, repeat until the sample becomes colorless. Continue with the test.
Fluoride	Interferes at all levels. See *Figure 1* below.
Iron	Greater than 20 mg/L
Phosphate	Greater than 50 mg/L
Polyphosphate	Polyphosphate interferes at all levels by causing negative errors and must not be present. Before running the test, polyphosphate must be converted to orthophosphate by acid hydrolysis as described under the phosphorus procedures.

Fluoride interferes at all levels by complexing with aluminum. The actual aluminum concentration can be determined using the Fluoride Interference Graph when the fluoride concentration is known.

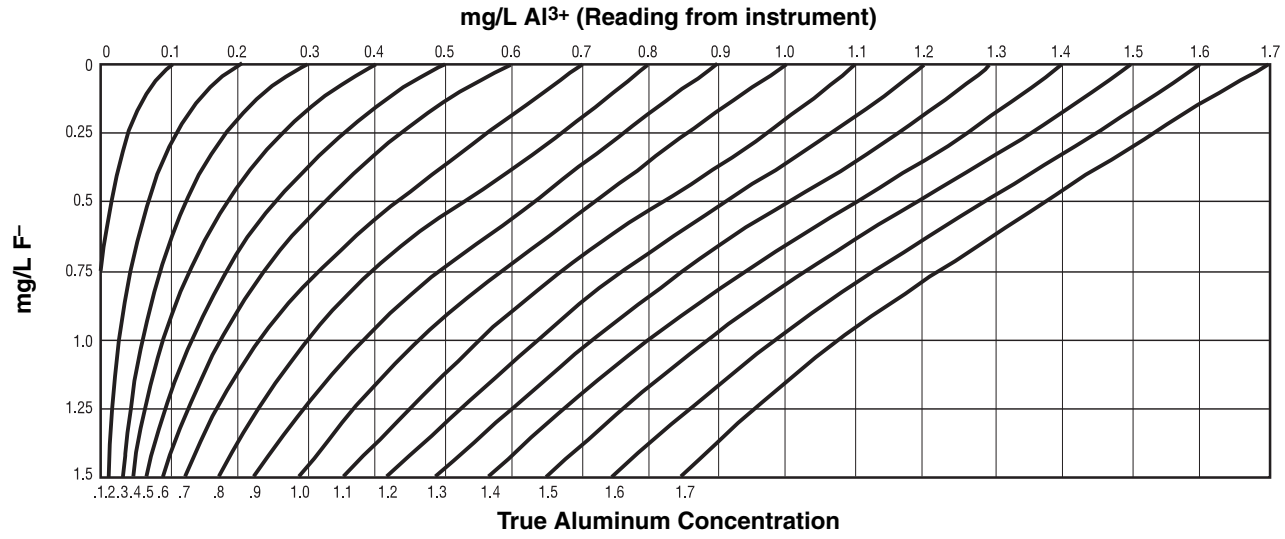
To use the fluoride interference graph:

1. Select the vertical grid line along the top of the graph that represents the aluminum reading obtained in *step 14*.

- Locate the point on the line where it intersects with the horizontal grid line that indicates how much fluoride is present in the sample.
- Extrapolate the true aluminum concentration by following the curved lines on either side of the intersect point down to the true aluminum concentration.

For example, if the aluminum test result was 0.7 mg/L Al and the fluoride present in the sample was 1 mg/L F^-, the point where the 0.7 grid line intersects with the 1 mg/L F^- grid line falls between the 1.2 and 1.3 mg/L Al curves. In this case, the true aluminum content would be 1.27 mg/L.

Figure 1 Fluoride Interference Graph



Sample Collection, Storage and Preservation

Collect samples in a clean glass or plastic containers. Preserve the sample by adjusting the pH to 2 or less with nitric acid (Cat. No. 152-49) (about 1.5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 3.5–4.5 with 5.0 N Sodium Hydroxide (Cat. No. 2450-26). Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open an Aluminum Voluette® Ampule Standard, 50-mg/L Al.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-41) with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 0.4-mg/L aluminum standard solution as follows: Pipet 1.00 mL of Aluminum Standard Solution, 100-mg/L as Al^{3+}, into a 250-mL volumetric flask.
2. Dilute to the mark with deionized water. Prepare this solution daily. Perform the aluminum procedure as described above. Go to *step 2*.

or

1. Using the TenSette Pipet, add 0.8 mL of solution from an Aluminum Voluette Ampule Standard Solution (50-mg/L as Al) into a 100-mL volumetric flask.
2. Dilute to volume with deionized water. Perform the aluminum procedure as described.
3. To adjust the calibration curve using the reading obtained with the 0.4-mg/L aluminum standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 0.40 mg/L Al^{3+}

Program	95% Confidence Limits of Distribution
10	0.383–0.417 mg/L Al^{3+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.008 mg/L Al^{3+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Aluminon indicator combines with aluminum in the sample to form a red-orange color. The intensity of color is proportional to the aluminum concentration. Ascorbic acid is added to remove iron interference. The AluVer 3 Aluminum Reagent, packaged in powder form, shows exceptional stability and is applicable for fresh water applications. Test results are measured at 522 nm.

Aluminum

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Aluminum Reagent Set (100 Tests)			22420-00
Includes:			
(1) AluVer® 3 Aluminum Reagent Powder Pillows	1 pillow	100/pkg	14290-99
(1) Ascorbic Acid Powder Pillows	1 pillow	100/pkg	14577-99
(1) Bleaching 3 Reagent Powder Pillows	1 pillow	100/pkg	14294-49
Hydrochloric Acid, 6.0 N	varies	500 mL	884-49
Water, deionized	varies	4 liters	272-56

Required Apparatus

Cylinder, graduated mixing, 50-mL, with glass stopper	1	each	1896-41
Sample Cell, 10-20-25 mL	2	6/pkg	24019-06

Required Standards

Aluminum Standard Solution, 100-mg/L as Al^{3+}		100 mL	14174-42
Aluminum Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as Al		16/pkg	14792-10
Nitric Acid Solution, 1:1		500 mL	2540-49
Water, deionized		4 liters	272-56

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WORLD HEADQUARTERS
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FAX: (970) 669-2932

3/04 1ed

Aluminum

Method 8326

Powder Pillows

Scope and Application: For water

Eriochrome Cyanine R Method*

(0.002 to 0.250 mg/L Al^{3+})

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Clean all glassware with 6.0 N HCl and deionized water before use to remove contaminants from the glass.
- The sample temperature must be between 20–25 °C (68–77 °F) for accurate results.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows — Method 8326

1. Touch

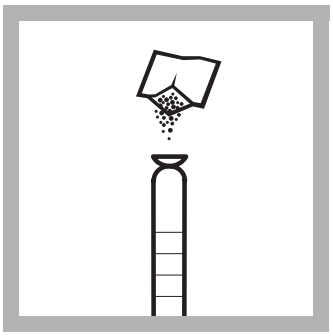
Hach Programs.

Select program

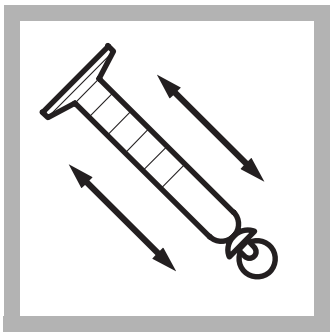
9 Aluminum, ECR.

Touch **Start.**

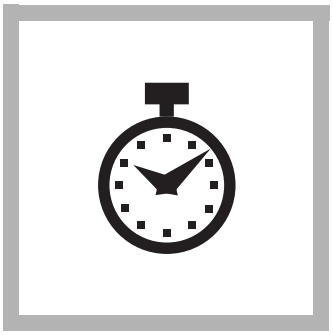
2. Fill a 25-mL graduated mixing cylinder to the 20-mL mark with sample.



3. Add the contents of one ECR Reagent Powder Pillow for 20-mL sample size.



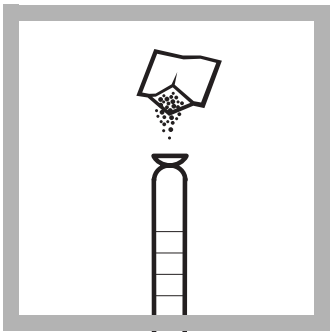
4. Stopper and invert several times to dissolve the powder.



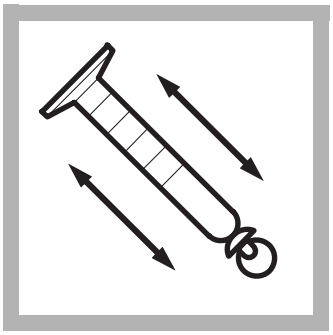
5. Touch the timer icon.

Touch **OK.**

A 30-second reaction period will begin.

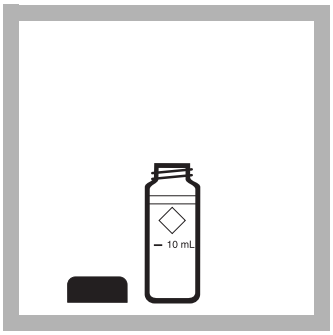


6. After 30 seconds add the contents of one Hexamethylene-tetramine Buffer Reagent powder pillow for 20-mL sample size.



7. Stopper and invert to mix until the powder is dissolved.

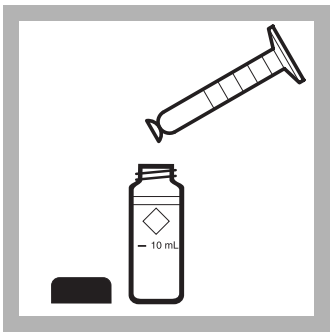
A red-orange color will develop if aluminum is present.



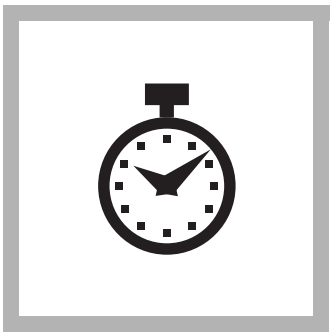
8. Put one drop of ECR Masking Reagent Solution into a clean, round sample cell. (This is the blank.)

9. Pour 10 mL from the mixing cylinder into the sample cell with the ECR Masking Reagent (the blank). Cap and invert to mix.

The solution will begin to turn yellow.



10. Fill a second round sample cell to the 10-mL mark with the remaining solution in the cylinder. (This is the prepared sample.)

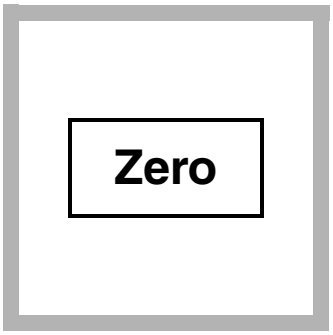


11. Touch the timer icon.

Touch **OK.**

A 5-minute reaction period will begin.

12. Within five minutes after the timer beeps, wipe the blank and place it into the cell holder.



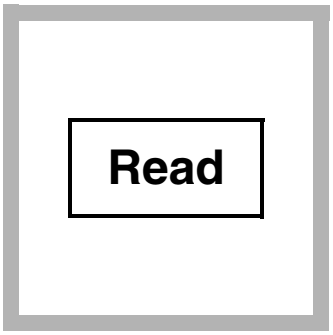
13. Touch **Zero**.

The display will show:

0.008 mg/L Al^{3+}

This test uses a non-zero intercept for the calibration curve.

14. Immediately wipe the prepared sample and place it into the cell holder.



15. Touch **Read**.

Results will appear in mg/L Al^{3+}.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 62 mg/L as $CaCO_2$
Alkalinity	Greater than 750 mg/L as $CaCO_3$
Ca^{2+}	Greater than 1000 mg/L as $CaCO_3$
Cl^-	Greater than 1000 mg/L
Cr^{6+}	0.2 mg/L (error is -5% of reading)
Cu^{2+}	2 mg/L (error is -5% of reading)
Fe^{2+}	Greater than 4 mg/L (error is positive and = mg/L Fe^{2+} x 0.0075)
Fe^{3+}	Greater than 4 mg/L (error is positive and = mg/L Fe^{3+} x 0.0075)
F^-	See table on page *5*.
Hexameta-phosphate	0.1 mg/L as PO_4^{3-} (error is -5% of reading)
Mg^{2+}	Greater than 1000 mg/L as $CaCO_3$
Mn^{2+}	Greater than 10 mg/L
NO_2^-	Greater than 5 mg/L
NO_3^-	Greater than 20 mg/L
pH	2.9–4.9 or 7.5–11.5. A sample pH between about 4.9 and 7.5 causes dissolved aluminum to partially convert to colloidal and insoluble forms. This method measures much of that hard-to-detect aluminum without any pH adjusting pretreatment as is necessary in some other methods.
PO_4^{3-} (ortho)	4 mg/L (error is -5% of reading)
Polyphosphate	See procedure below.
SO_4^{2-}	Greater than 1000 mg/L
Zn^{2+}	Greater than 10 mg/L

Polyphosphate interference can be reduced by converting polyphosphate to orthophosphate by the following steps:

a. Rinse a 50-mL graduated mixing cylinder and a 125-mL Erlenmeyer flask containing a magnetic stir bar with 6 N hydrochloric acid (Cat. No. 884-49). Rinse again with deionized water. This will remove any aluminum present.

Note: *Rinse two Erlenmeyer flasks if a reagent blank is used; see* step b *below.*

b. Measure 50 mL deionized water into the 125-mL Erlenmeyer flask using the graduated cylinder. This is the reagent blank. Because of the test sensitivity, this step must be done only when any of the reagents used in the following pretreatment are replaced—even if the new reagent has a matching lot number. When the pretreated sample has been analyzed, correct for the aluminum concentration of the reagent blank by touching **Options**, then **Reagent Blank**. Touch **On**. Enter the reagent blank value and touch **OK**.

c. Measure 50 mL sample into the 125-mL Erlenmeyer flask using the graduated cylinder. Use a small amount of deionized water to rinse the cylinder contents into the flask.

d. Add 4.0 mL of 5.25 N Sulfuric Acid Standard Solution (Cat. No. 2449-32).

e. Use a combination hot plate/stirrer to boil and stir the sample for at least 30 minutes. Add deionized water as needed to maintain a sample volume of 20-40 mL. Do not boil dry.

f. Cool the solution to near room temperature.

g. Add 2 drops of Bromphenol Blue Indicator Solution (Cat. No. 14552-32).

h. Add 1.5 mL of 12.0 N Potassium Hydroxide Standard Solution (Cat. No. 230-32) using the calibrated, plastic dropper provided. Swirl to mix. The solution color should be yellow or green but not purple. If the color is purple, begin with *step a* again using an additional 1 mL Sulfuric Acid Standard Solution in *step d*.

i. While swirling the flask, add 1.0 N Potassium Hydroxide Solution (Cat. No. 23144-26), a drop at a time, until the solution turns a dirty green color.

j. Pour the solution into the graduated cylinder. Rinse the flask contents into the graduated cylinder with deionized water (Cat. No. 272-56) to bring the total volume to 50 mL.

k. Use this solution in *step 3* of the ECR method.

Fluoride interference can be corrected by using the table on page *5*.

Example:

If the fluoride concentration is known to be 1.00 mg/L F^- and the ECR method gives a reading of 0.060 mg/L aluminum, what is the true mg/L aluminum concentration?

Intermediate values can be found by interpolation. Do not use correction graphs or charts found in other publications.

Answer: 0.183 mg/L

DR/2400 Reading	Fluoride Concentration (mg/L)									
(mg/L)	0.00	0.20	0.40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.010	0.019	0.030	0.040	0.052	0.068	0.081	0.094	0.105	0.117	0.131
0.020	0.020	0.032	0.046	0.061	0.077	0.099	0.117	0.137	0.152	0.173	0.193
0.030	0.030	0.045	0.061	0.077	0.098	0.124	0.146	0.166	0.188	0.214	0.243
0.040	0.040	0.058	0.076	0.093	0.120	0.147	0.174	0.192	0.222		
0.050	0.050	0.068	0.087	0.109	0.135	0.165	0.188	0.217			
0.060	0.060	0.079	0.100	0.123	0.153	0.183	0.210	0.241			
0.070	0.070	0.090	0.113	0.137	0.168	0.201	0.230				
0.080	0.080	0.102	0.125	0.152	0.184	0.219					
0.090	0.090	0.113	0.138	0.166	0.200	0.237					
0.100	0.100	0.124	0.150	0.180	0.215						
0.120	0.120	0.146	0.176	0.209	0.246						
0.140	0.140	0.169	0.201	0.238							
0.160	0.160	0.191	0.226								
0.180	0.180	0.213									
0.200	0.200	0.235									
0.220	0.220										
0.240	0.240	True Aluminum Concentration (mg/L) Al									

Sample Collection, Storage and Preservation

Collect samples in a clean glass or plastic containers. Preserve samples by adjusting the pH to 2 or less with concentrated nitric acid (about 1.5 mL per liter) (Cat. No. 2540-49). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 2.9–4.9 with 12.0 N Potassium Hydroxide Standard Solution (Cat. No. 230-32) and/or 1 N Potassium Hydroxide Solution (Cat. No. 23144-26). Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Solution Method

Prepare a 0.100 mg/L aluminum standard solution as follows:

1. Using Class A glassware, pipet 1.00 mL of Aluminum Standard Solution, 100-mg/L as Al^{3+}, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the aluminum procedure as described above.

   Or, add 2.0 mL of solution from an Aluminum Voluette® Ampule Standard Solution (50-mg/L as Al) into a 1000-mL volumetric flask. Dilute to volume with deionized water. Prepare this solution daily. Perform the aluminum procedure as described above.

2. To adjust the calibration curve using the reading obtained with the 0.100-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.

3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the **Adjust to** field and enter the actual concentration. Press **OK**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.100 mg/L Al^{3+}

Program	95% Confidence Limits of Distribution
9	0.086–0.114 mg/L Al^{3+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.002 mg/L Al^{3+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Eriochrome Cyanine R combines with aluminum in a sample to produce an orange-red color. The intensity of color is proportional to the aluminum concentration. Test results are measured at 535 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Aluminum Reagent Set (100 Tests)			26037-00
Includes:			
ECR Reagent Powder Pillows	1 pillow	100/pkg	26038-49
Hexamethylenetetramine Buffer Reagent Powder Pillows	1 pillow	100/pkg	26039-99
ECR Masking Reagent Solution	2 drops	25 mL SCDB	23801-23

Required Apparatus

Cylinder, 25-mL, graduated mixing, with glass stopper	1	each	1896-40
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Thermometer, -10 to 110 °C		each	1877-01

Required Standards

Aluminum Standard Solution, 100-mg/L		100 mL	14174-42
Aluminum Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as Al		16/pkg	14792-10
Water, deionized		4 liters	272-56

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Aluminum

Chromazurol S Method

UniCell™ Vials **(0.02 to 0.50 mg/L Al^{3+})**

Scope and Application: For drinking water, swimming pool water, wastewater, and process control

Tips and Techniques

- Analyze collected samples in glass or plastic bottles as quickly as possible.
- Adjust pH of preserved samples to between pH 2–3 with 5.0 N Sodium Hydroxide Standard Solution before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check*.
- See *Section 3.2.4 Adjusting the Standard Curve* on page *40* in the procedure manual for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is between 18–22 °C
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

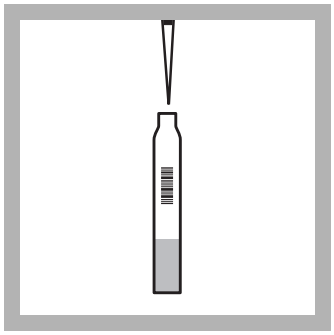
1. Touch

Hach Programs.

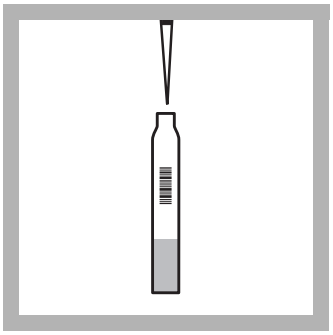
Select program

12 Aluminum, HCT 150

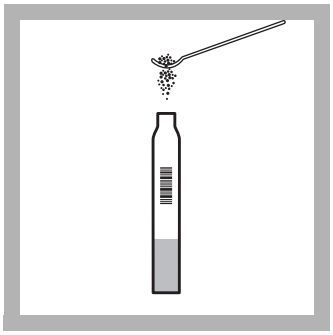
Touch **Start**.



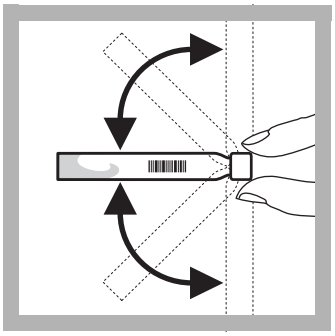
2. Pipet 2 mL of Buffer Solution A (HCT 150 A) into a sample vial.



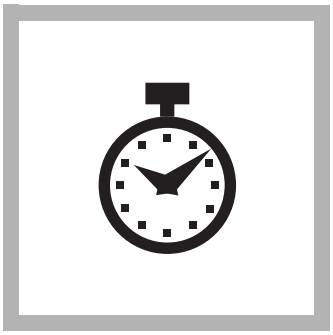
3. Pipet 3 mL of sample into the sample vial.



4. Use the spoon to add one level spoonful of Masking Agent B (HCT 150 B) to the sample vial.



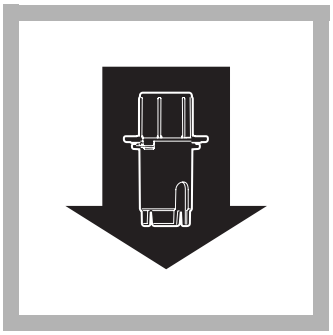
5. Cap the vial. Invert the sample until the solids dissolve completely.



6. Touch the timer icon.

Touch **OK**.

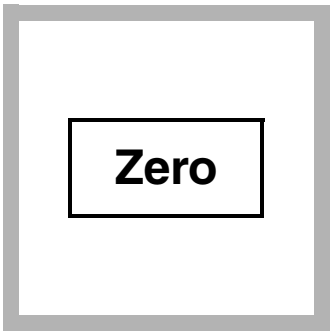
A 25-minute reaction period will begin.



7. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

When the timer beeps, wipe the zero vial (**white** cap) and place it into the cell adapter.

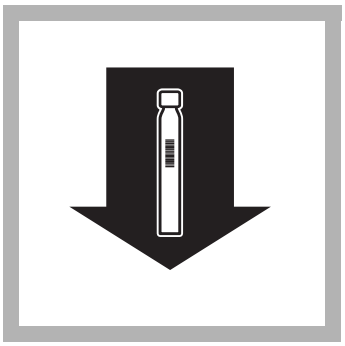


8. Touch **Zero**.

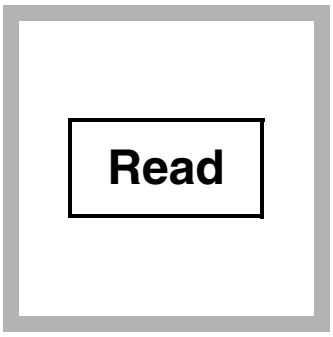
The display will show:

0.00 mg/L Al^{3+}

Underrange



9. Wipe the sample vial and place it into the cell adapter.



10. Touch **Read**.

Results will appear in mg/L Al^{3+}.

Interference

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Mg^{2+}, K^{+}, Na^{+}, NH_4^{+}, Cl^{-}, NO_3^{-}, Ca^{2+}	500 mg/L
Ag^{+}, Mn^{2+}	100 mg/L
Cd^{2+}, Co^{2+}, Ni^{2+}, Sn^{2+}, Pb^{2+}, PO_4^{3-}	50 mg/L
Cu^{2+}, Hg^{2+}	10 mg/L
Fe^{2+}, Fe^{3+}, Zn^{2+}, Si^{4+}	5 mg/L

(continued)

Interfering Substance	Interference Levels
Cr^{3+}, Cr^{6+}	0.5 mg/L
F^-	0.1 mg/L

Higher concentrations of heavy metals than those given, as well as fluoride, phosphate and relatively rare elements such as beryllium, thorium, titanium, zirconium and vanadium interfere with the determination. Aluminum oxide hydrates and hydroxide are only partially determined.

Note: *Sample pretreatment with the Metal Prep Set (HCT 200) will cause an interference.*

Sample Collection, Preservation, and Storage

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 2.0 and 3.5 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 3.5 or aluminum may precipitate or complex. The temperature of the water sample and the sample vial should be 18–22 °C (64–72 °F).

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Measure 250 mL of sample into three graduated cylinders.
5. Pipet 0.1, 0.2, and 0.3 mL of 100-mg/L Al standard solution, respectively, into the three cylinders and mix thoroughly.
6. Analyze as described in the procedure. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 0.30-mg/L Al standard solution by pipetting 0.30 mL of 100-mg/L Al standard into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Analyze using the procedure described above.

2. To adjust the calibration curve using the reading obtained with the 0.30-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision

Standard: 0.30 mg/L Al^{3+}

Program	95% Confidence Limits of Distribution
12	0.25–0.35 mg/L Al^{3+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
12	Entire range	0.010	0.007 mg/L Al^{3+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chromazurol S forms a green complex with aluminum in a slightly acidic acetate buffer. Measurements are taken at 620 nm.

Required Reagents

Description	Unit	Cat. No.
Aluminum - Al, UniCell™ HCT 150	23/pkg	HCT 150

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Flask, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 250-mL	each	20886-46
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pk/100	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	pk/400	27950-00
pH Paper	pk/100	26013-00

Optional Reagents

Aluminum Standard Solution, 100-mg/L Al	100 mL	14174-42
Nitric Acid, concentrated	500 mL	152-49
Sodium Hydroxide Standard Solution, 5.0 N	1000 mL	2450-53

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

★Method 8013 Silver Diethyldithiocarbamate Method* (0 to 0.200 mg/L As)

Scope and Application: For water, wastewater, and seawater; distillation is required; USEPA accepted** for reporting for drinking and wastewater analysis (digestion required, see *Section 4* on page *55* for the digestion procedure.)

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

** Procedure is equivalent to USEPA Method 206.4 for wastewater and Standard Method 3500-As for drinking water analysis.

Tips and Techniques

- This procedure requires a user-entered calibration for each new lot of arsenic absorber solution. See *Calibration on page 5*.
- Some variations of the calibration procedure are possible. See the *Instrument Manual* for details.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- The arsenic absorber in this test is a silver solution in pyridine. Both silver (D011) and pyridine (D038) are regulated by the Federal RCRA as hazardous waste. In addition, the cotton ball soaked in lead acetate (D008) solution is a hazardous waste. These materials should not be poured down the drain. See *Section 4* of this manual for more information on proper disposal of these materials.

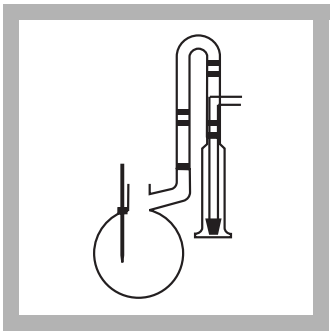
Method 8013

1. Perform the *User Programming* procedure on page *5*.

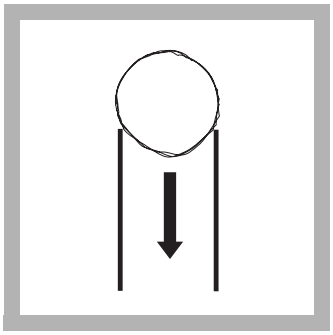
2. Touch **User Program**.

Select the program number for arsenic.

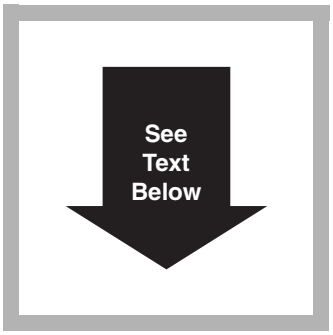
Touch **Start.**



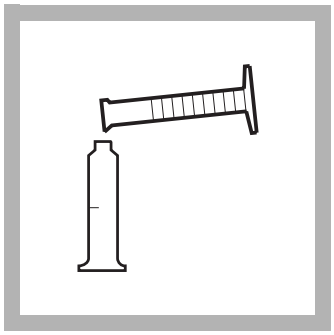
3. Prepare the Hach Distillation Apparatus for arsenic recovery. See the *Hach Distillation Manual* for assembly instructions. Place it under a fume hood to vent toxic fumes.



4. Dampen a cotton ball with 10% Lead Acetate Solution. Place it in the gas scrubber. Be certain that the cotton seals against the glass.

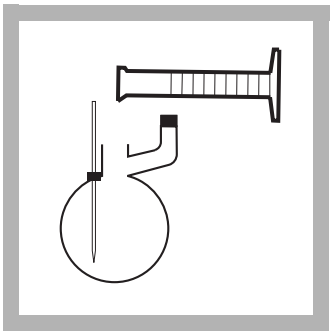


5. Prepare the arsenic absorber solution as directed under *Reagent Preparation* on page 4.



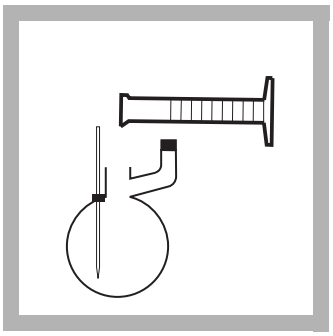
6. Using a graduated cylinder, pour 25-mL of prepared arsenic absorber solution into the cylinder/gas bubbler assembly.

Attach it to the distillation apparatus.

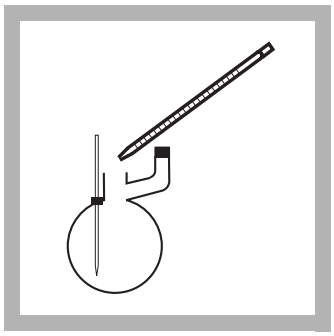


7. Using a graduated cylinder, pour 250 mL of sample into the distillation flask.

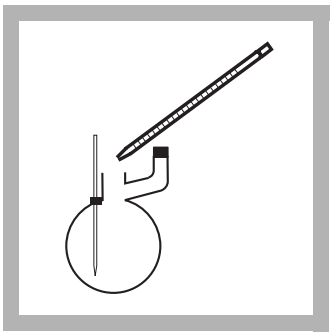
8. Turn on the power switch. Set the stir control to 5. Set the heat control to 0.



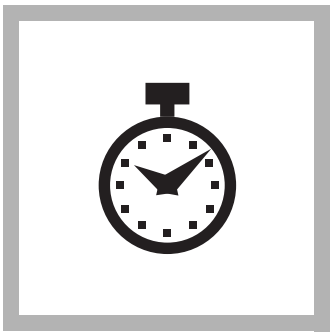
9. Using a graduated cylinder, add 25 mL of Hydrochloric Acid, ACS, to the distillation flask.



10. Use a serological pipet to add 1 mL of Stannous Chloride Solution to the flask.



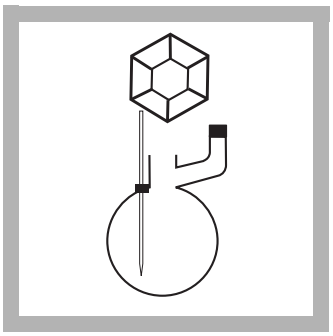
11. Use a serological pipet to add 3 mL of Potassium Iodide Solution to the flask. Cap.



12. Touch the timer icon.

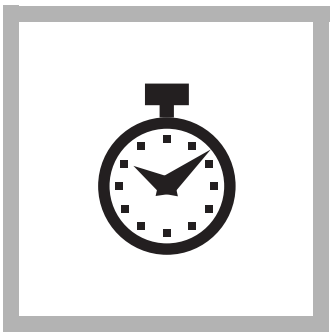
Touch **OK**.

A 15-minute reaction period will begin.



13. When the timer beeps, add 6.0 g of 20-mesh zinc to the flask. Cap immediately.

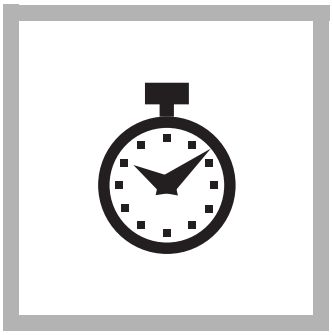
14. Set the heat control to 3.



15. Touch the timer icon.

A second 15-minute reaction period will begin.

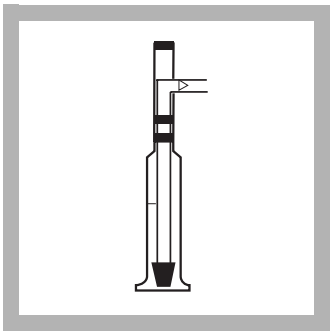
16. When the timer beeps, set the heat control to 1.



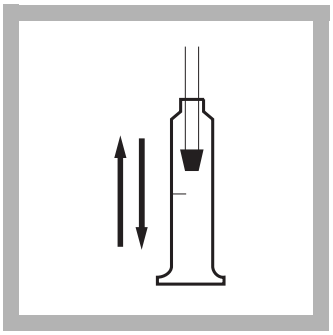
17. Touch the timer icon.

Touch **OK**.

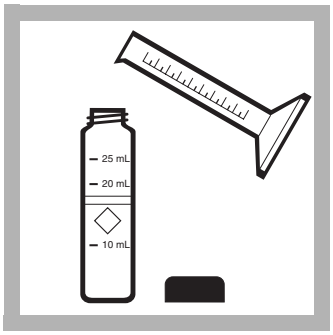
A third 15-minute reaction period will begin.



18. When the timer beeps, turn off the heater. Remove the cylinder/gas bubbler assembly as a unit.

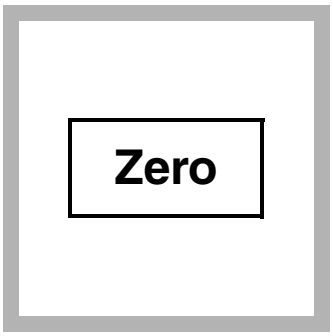


19. Rinse the gas bubbler by moving it up and down in the arsenic absorber solution.



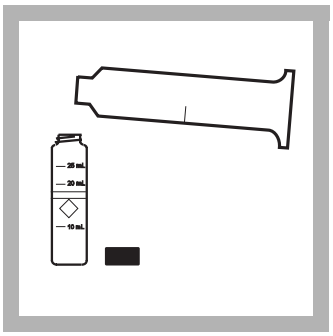
20. Fill a dry, 25-mL sample cell with untreated arsenic absorber solution (the blank). Stopper.

21. Wipe the blank and place it into the cell holder.



22. Touch **Zero**.

The display will show the intercept as calculated from your user-entered calibration curve. This will probably be a non-zero intercept.



23. Pour the reacted arsenic absorber sample into a sample cell. If the solution volume is less than 25 mL, add pyridine to bring the volume to exactly 25 mL. Stopper.

24. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Interferences

Interfering Substance	Interference Levels and Treatments
Antimony Salts	May interfere with color development.

Sample Collection, Storage and Preservation

Collect samples in acid washed glass or plastic bottles. Adjust the pH to 2 or less with sulfuric acid (about 2 mL per liter) (Cat. No. 1270-32). Preserved samples may be stored up to six months at room temperature. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Reagent Preparation

Prepare the arsenic absorber solution as follows:

1. Weigh 1.00 g of silver diethyldithiocarbamate on an analytical balance.
2. Transfer the powder to a 200-mL volumetric flask. Dilute to volume with pyridine. You must use pyridine only in a fume hood and wear chemical resistant gloves. Read the MSDS before using pyridine.
3. Mix well to dissolve. Store the reagent, tightly sealed, in an amber bottle. The reagent is stable for one month if stored in this manner. Larger volumes of reagent can be prepared if the reagent is used within one month.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare a 100-mg/L arsenic working standard by pipetting 10.0 mL of Arsenic Standard Solution, 1000-mg/L As (Cat. No. 14571-42) into a 100-mL volumetric flask. Dilute to volume with deionized water.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 250 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

To check accuracy, use a 0.100 mg/L arsenic standard solution prepared as follows:

1. Pipet 1 mL of a 1000-mg/L Arsenic Standard Solution into a 100-mL Class A volumetric flask. Dilute to the mark with deionized water and mix thoroughly.
2. Pipet 10 mL of the solution prepared in step a into a 1 L class A volumetric flask. Dilute to the mark with deionized water and mix thoroughly. This is a 0.100-mg/L arsenic standard solution.

Calibration

Standard Preparation

Perform a new calibration for each lot of arsenic absorber solution.

1. Prepare a 10.0-mg/L arsenic working standard by pipetting 10.0 mL of Arsenic Standard Solution, 1000 mg/L As (Cat. No. 14571-42) into a 1000-mL volumetric flask.
2. Dilute to volume with deionized water.
3. Into three different 500-mL volumetric flasks, pipet 1.0, 2.0, and 10.0 mL of the 10.0 mg/L As stock solution using Class A glassware.
4. Dilute to the mark with deionized water and mix thoroughly. These standards have concentrations of 0.02, 0.04 and 0.20 mg/L As.

User Programming

1. Touch **User Programs** on the main menu.
2. If you have not performed an arsenic calibration before, touch **Program Options** and **New Program**. Key any available program number (950–999) to use for arsenic testing. Touch **OK**. You are now in the *Edit Program* mode. Fill in the appropriate fields using the touch screen by touching **Edit** when the field is highlighted. Use the alphanumeric keys to name your User Program **Arsenic**. Set up the rest of the parameters as follows:

Units	mg/L	**Upper Limit**	0.220 mg/L
Resolution	0.001	**Lower Limit**	-0.020 mg/L
Wavelength	520 nm	**Timer1**	15 minutes
Timer2	15 minutes	**Timer3**	15 minutes

   If you already have a working arsenic program, highlight **Program Options,** then touch **Edit Program**.
3. Touch **Calibration: C = a + bA**.
4. Touch **Edit**. Touch **Curve Fit**. Touch **+**.
5. Enter the mg/L value of the sample to be read and touch **OK**.
6. Place the 25-mL sample cell containing only unreacted arsenic absorber solution into the cell holder. Touch **Zero.**
7. Place the prepared sample in the cell holder. Touch **Read** to accept the absorbance value. Repeat steps *4* through *6* for each standard.
8. Touch **Graph**. Make sure **Force Zero** is off.
9. If the graph is acceptable touch **Done**.
10. Touch **OK**. Touch **Save Program**. Touch **Done**.

The program is ready for use.

Some variations of the calibration procedure are possible. See the *Instrument Manual* for details.

Summary of Method

Arsenic is reduced to arsine gas by a mixture of zinc, stannous chloride, potassium iodide, and hydrochloric acid in a specially equipped distillation apparatus. The arsine is passed through a scrubber containing cotton saturated with lead acetate and then into an absorber tube containing silver diethyldithiocarbamate in pyridine. The arsenic reacts to form a red complex which is read colorimetrically. This procedure requires a manual calibration. Test results are measured at 520 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Arsenic Standard Solution, 1000-mg/L As	varies	100 mL	14571-42
Hydrochloric Acid, ACS	25 mL	500 mL	134-49
Lead Acetate Solution, 10%	1 mL	100 mL	14580-42
Potassium Iodide Solution, 20%	3 mL	100 mL	14568-42
Pyridine, ACS	50 mL	500 mL	14469-49
Silver Diethyldithiocarbamate	1 g	25 g	14476-24
Stannous Chloride Solution	1 mL	100 mL	14569-42
Water, deionized		4 liters	272-56
Zinc, 20-mesh, ACS	6 g	454 g	795-01

Required Apparatus

Balance, analytical, SA-120, 115 VAC	1	each	26103-00
Balance, analytical, SA-120, 220 VAC	1	each	26103-01
Balls, cotton	1	100/pkg	2572-01
Boat, weighing, 8.9-cm square	2	500/pkg	21790-00
Bottle, amber, 237-mL, glass	1	6/pkg	7144-41
Cap, polypropylene, for amber bottle	1	6/pkg	21667-06
Cylinder, graduated, 25-mL	2	each	508-40
Cylinder, graduated, 250-mL	1	each	508-46
Distillation Apparatus, Arsenic Accessories	1	set	22654-00
Distillation Apparatus, General Purpose Accessories	1	set	22653-00
Flask, volumetric, Class A, 1000-mL, with glass stopper	1	each	14574-53
Flask, volumetric, Class A, 200-mL	1	each	14574-45
Flask, volumetric, Class A, 500-mL	6	each	14574-49
Pipet Filler, safety bulb	1	each	14651-00
Pipet, serological, 5-mL	2	each	532-37
Pipet, volumetric, Class A, 1.00-mL	2	each	14515-35
Pipet, volumetric, Class A, 2.00-mL	1	each	14515-36
Pipet, volumetric, Class A, 4.00-mL	1	each	14515-04
Pipet, volumetric, Class A, 6.00-mL	1	each	14515-06
Pipet, volumetric, Class A, 8.00-mL	1	each	14515-08
Pipet, volumetric, Class A, 10.00-mL	1	each	14515-38
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Stopper, hollow, poly., No. 1	2	6/pkg	14480-00
Select one based on available voltage:			
Distillation Apparatus Heater, 115 VAC, 60 Hz		each	22744-00
Distillation Apparatus Heater, 230 VAC, 50 Hz		each	22744-02

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Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Method 10050 **Immunoassay Method***

Scope and Application: For water

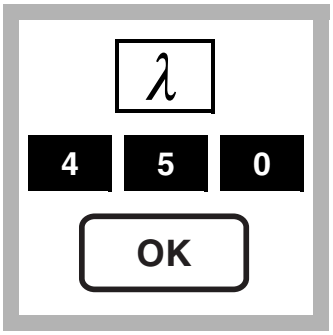
* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

This method analyzes for Atrazine in water. Sample calibrators and reagents are added to cuvettes coated with Atrazine-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

Tips and Techniques

- Read the entire procedure before starting. Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis. A 1-cm square cell holder is required for this procedure.
- Timing is critical; follow instructions carefully.
- A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the spectrophotometer.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
- Hach Company recommends wearing protective nitrile gloves for this procedure.

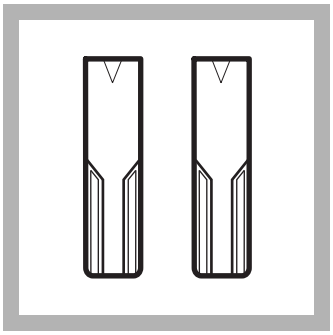
Immunoassay Procedure for Water



1. Touch

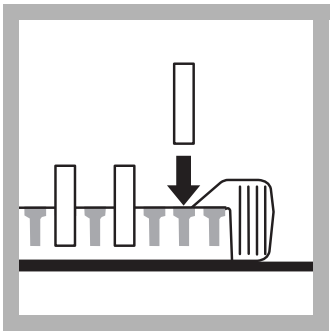
Single Wavelength

then touch the λ button. Type in 450 nm and touch **OK.**

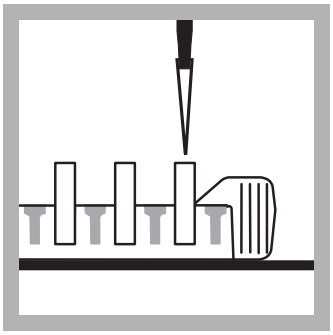


2. Label an Antibody Cuvette for each calibrator and each sample to be tested.

Note: *As many as 20 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.*

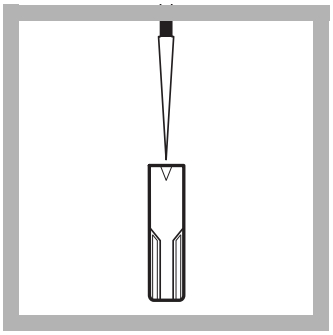


3. Place the cuvettes into the rack snugly.



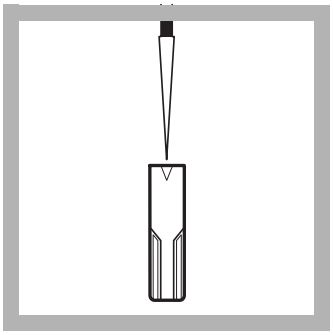
4. Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

Note: *Use a new pipette tip for each calibrator.*

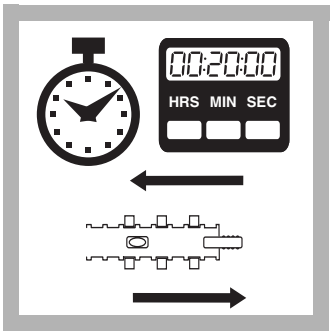


5. Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

Note: *Use a new pipette tip for each sample.*



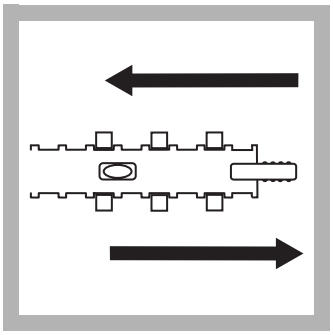
6. Immediately pipet 0.5 mL of Atrazine Enzyme Conjugate into each cuvette.



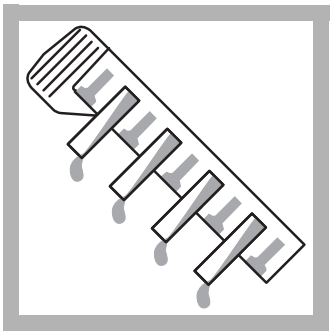
7. Touch the timer icon. Enter 20 minutes and touch **OK.**

A 20-minute reaction time will begin.

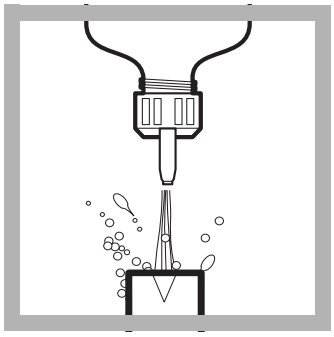
Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack on page 5*.



8. After 10 minutes mix the contents of the rack for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack on page 5*.



9. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

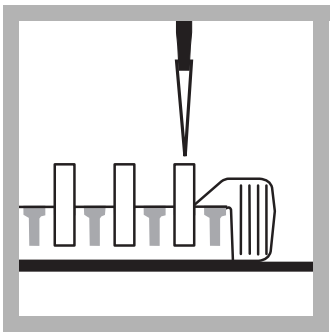


10. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: *Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.*

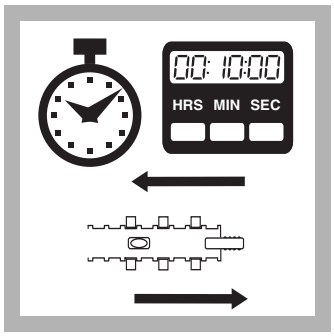
Color Development

Note: *Timing is critical; follow instructions carefully.*



11. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: *Use a new pipette tip for each cuvette.*

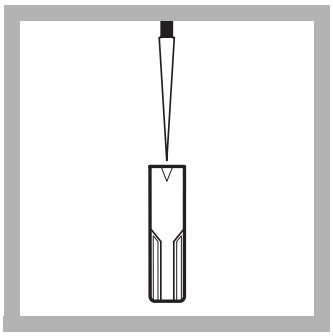


12. Touch the timer icon. Enter 10 minutes and touch **OK.**

A reaction period will begin. Mix, using the instructions on page 5 in *Using the 1-cm MicroCuvette Rack*.

13. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Note: *Solutions will turn blue in some or all of the cuvettes.*



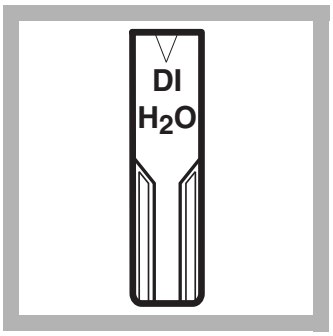
14. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in *step 11*.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

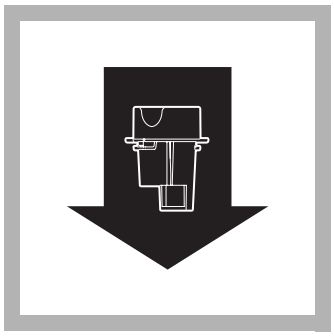
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

Note: *The same pipette tip can be used repeatedly for this step.*

Measuring the Color



15. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

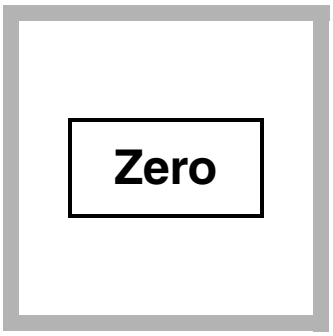


16. Install the 1-cm square cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Place the filled zeroing cuvette into the cell holder—arrow pointing towards the left side of the instrument.

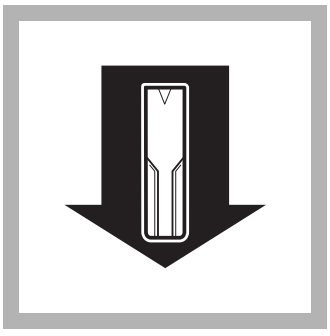
Orient the arrow in the same direction for all cuvettes.



17. Touch **Zero**.

The display will show:

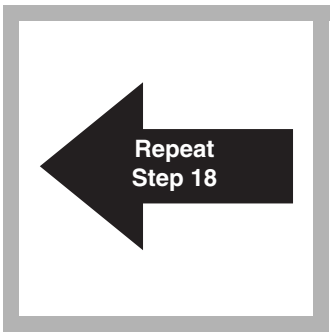
0.000 Abs



18. Place the first calibrator into the celladapter.

Touch **Read**.

The display will give an absorbance reading. Record the results for each calibrator and sample.



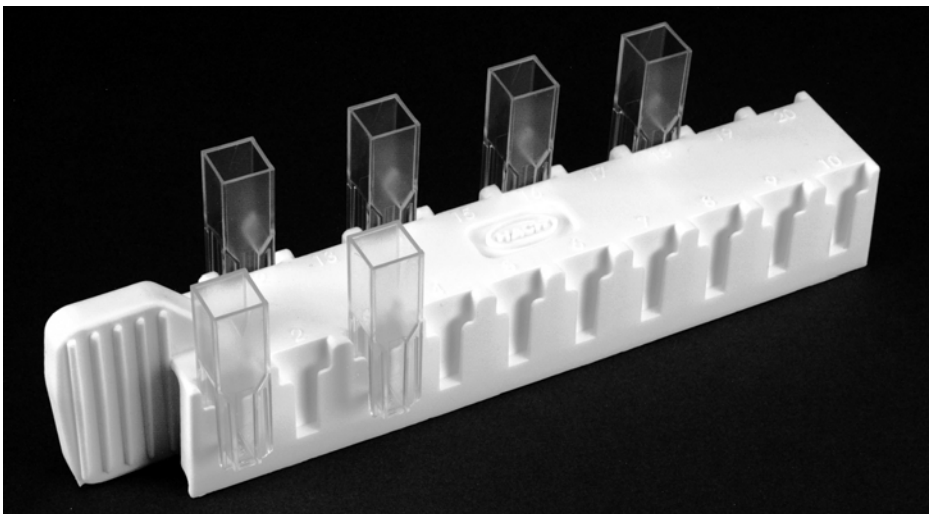
19. Repeat *step 18* for all remaining calibrators and samples.

See *Interpreting and Reporting Results* for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 2*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 2 The 1-cm MicroCuvette Rack



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of Atrazine and the reading. In other words, the higher the reading, the lower the concentration of Atrazine.

If the sample reading is…	the sample Atrazine Concentration is…
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example

Readings:

0.5 ppb Atrazine Calibrator: **0.475 Abs**

3.0 ppb Atrazine Calibrator: **0.245 Abs**

Sample #1: **0.140 Abs**

Sample #2: **0.300 Abs**

Sample #3: **0.550 Abs**

Interpretation

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Atrazine is greater than both 0.5 ppb and 3.0 ppb Atrazine.

Sample #2 — Sample reading is between the readings for the 0.5 ppb and 3.0 ppb Atrazine calibrators. Therefore the sample concentration of Atrazine is between 0.5 ppb and 3.0 ppb.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Atrazine is less than both 3.0 ppb and 0.5 ppb.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Atrazine Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Atrazine immunoassay cannot differentiate between the various triazines and metabolites, but it detects their presence in differing degrees.

Table 1 Required Concentrations for Selected Chemicals

Compound	Concentration to give a positive result at 3 ppb (in ppb)
Ametryne	1
Atrazine	3
Atrazine, de-ethylated	115
Atrazine, de-isopropyl	714
Cyanazine	460
Cyromazine	1200
Prometon	8
Prometryne	0.7
Propazine	2.3
Simetryne	5.4
Simazine	37
Terbuthylazine	91
Terbutryne	8.3

The following compounds are not detectable at 10,000 ppb.

Alachlor	Carbofuran	Metaolachlor
Aldicarb	Diaminoatrazine	2,4-D
Carbendazim	Melamine	

Sample Collection and Storage

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Atrazine-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Atrazine from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Atrazine compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Atrazine and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Atrazine in the sample. The resulting color is then compared with a calibrator to determine whether the Atrazine concentration in the sample is greater or less than the threshold levels. Test results are measured at 450 nm.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, Atrazine*	20 cuvettes	27627-00

Required Apparatus

Adapter, 1-cm square cell	each	59459-00
Caps, flip spout	2/pkg	25818-02
Cell holder, 1-cm square	each	59065-00
Marker, laboratory	each	20920-00
Rack, for 1-cm Micro Cuvettes	each	48799-00
Wipes, disposable	box	20970-00
TenSette®, Pipet, 0.1–1.0 mL	each	19700-01
Tips, for pipettor 19700-01	1000/pkg	21856-28

Optional Reagents

Reagent Set, Atrazine	100 cuvettes	27627-10

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.

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ATZ_None_Other_IMM_Eng_Ody.fm

Method 8014

Turbidimetric Method*

Powder Pillows or AccuVac® Ampuls

(1 to 100 mg/L)

Scope and Application: For water, wastewater, oil-field water, and seawater

* Adapted from Snell and Snell, *Colorimetric Methods of Analysis, Vol. II*, 769 (1959).

Tips and Techniques

- Perform a standard curve adjustment or a new calibration for each new lot of reagent. See *Standard Solutions* and *Calibration Standard Preparation*.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Filter highly colored or turbid water samples using a funnel (Cat. No. 1083-67) and filter paper (Cat. No. 1894-57). Large amounts of color or turbidity will interfere and cause high readings.
- Immediately after each test, clean the sample cell with soap, water, and a brush to prevent a film of barium from forming inside the sample cell.

Powder Pillows — Method 8014

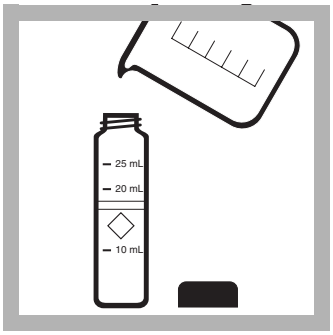
1. Touch

Hach Programs.

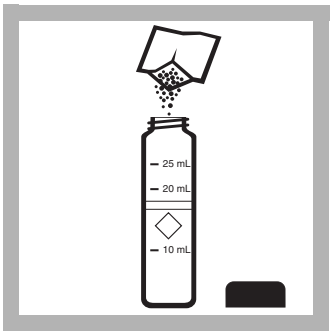
Select program

20 Barium

Touch **Start**.



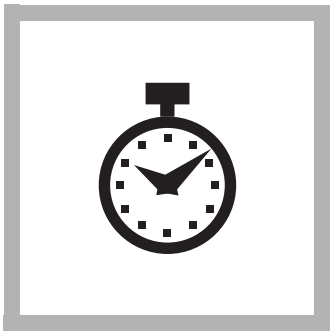
2. Fill a round sample cell with 25 mL of sample.



3. Add the contents of one BariVer® 4 Barium Reagent Powder Pillow to the cell (this is the prepared sample).

Cap and invert to mix.

Note: *If barium is present, a white turbidity will develop.*

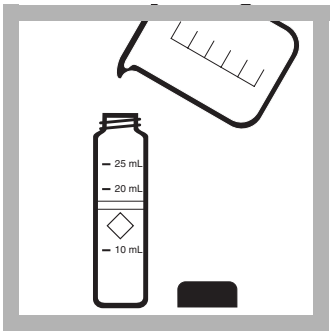


4. Touch the timer icon.

Touch **OK**.

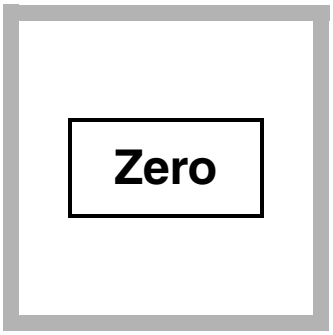
A five-minute reaction period will begin.

Do not disturb the sample during the reaction period.



5. Fill another sample cell with 25 mL of sample (this is the blank).

6. When the timer beeps, wipe the blank and place it into the cell holder.



7. Touch **Zero**.

The display will show:

0 mg/L Ba^{2+}

8. Within 10 minutes after the timer beep, wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L Ba^{2+}.

AccuVac Ampul — Method 8014

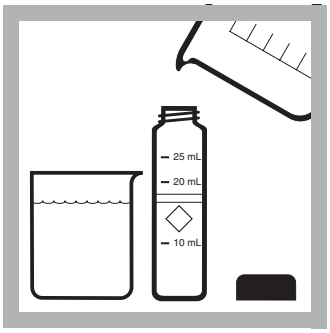
1. Touch

Hach Programs.

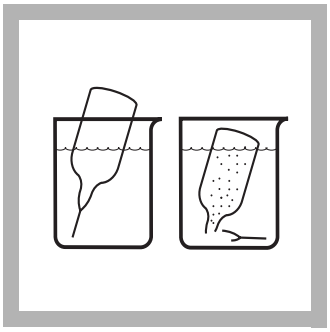
Select program

25 Barium, AV

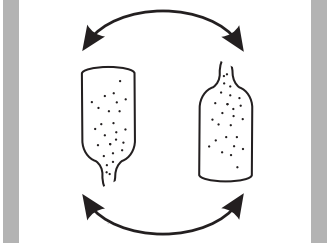
Touch **Start**.



2. Fill a round sample cell with 25-mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.

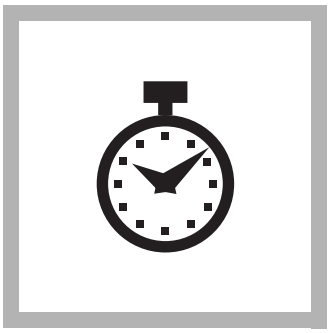


3. Fill a barium AccuVac® Ampul with sample. (This is the prepared sample.) Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampule several times to mix, then wipe off any liquid or fingerprints.

If barium is present, a white turbidity will develop.



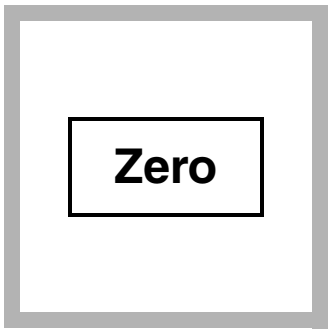
5. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

Do not disturb the sample during the reaction period.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0 mg/L Ba^{2+}

8. Within five minutes after the timer beep, place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L Ba^{2+}.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	10,000 mg/L as $CaCO_3$
Magnesium	100,000 mg/L as $CaCO_3$
Silica	500 mg/L
Sodium Chloride	130,000 mg/L as NaCl
Strontium	Interferes at any level. If present, the total concentration between barium and strontium may be expressed as a PS (Precipitated by Sulfate). While this does not distinguish between barium and strontium, it gives an accurate indication of scaling tendency.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment (see *Section 3.3 Interferences* on page *41)*.

Sample Collection, Storage, and Preservation

Collect samples in an acid cleaned glass or plastic container. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter) (Cat. No. 2540-49). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 5 with 5.0 N sodium hydroxide (Cat. No. 2040-53). Correct the test result for volume additions (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).

Standard Solutions

Prepare a 90.0-mg/L barium standard solution as follows:

1. Pipet 9.00 mL of Barium Standard Solution, 1000-mg/L, into a 100-mL volumetric flask.
2. Dilute to the mark with deionized water.
3. Prepare this solution daily. Perform the barium procedure as described above.

To adjust the calibration curve using the reading obtained with the 90.0-mg/L standard solution:

1. Touch **Options** on the current program menu. Touch **Standard Adjust** on the Options menu.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the unspiked sample in the instrument. Verify that the units displayed are in mg/L.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a Barium Standard Solution, 1000-mg/L Ba.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Touch the timer icon. After the timer beeps, read the result.
6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Touch the timer icon. After the timer beeps, read the result.
7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Touch the timer icon. After the timer beeps, read the result. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac Ampuls, fill three mixing cylinders (Cat. No. 1896-41) with 50 mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Standard: 30 mg/L Ba

Program	95% Confidence Limits of Distribution
20	25–35 mg/L Ba
25	25–35 mg/L Ba

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
20	Entire Range	0.010	1 mg/L Ba
25	Entire Range	0.010	1 mg/L Ba

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Calibration Standard Preparation

Prepare calibration standard containing 10, 20, 30, 50, 80, 90, and 100 mg/L Ba as follows:

1. Into seven different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 5, 8, 9 and 10 mL of the 1000-mg/L Barium Standard Solution using Class A glassware.
2. Dilute to the mark with deionized water. Mix thoroughly.
3. Using the turbidimetric method and the calibration procedure described in the *User-Entered Programs* section of the instrument manual, generate a calibration curve from the standards prepared above.

Summary of Method

The BariVer® 4 Barium Reagent Powder combines with barium to form a barium sulfate precipitate, which is held in suspension by a protective colloid. The amount of turbidity present caused by the fine white dispersion of particles is directly proportional to the amount of barium present. Test results are measured at 450 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
BariVer® 4 Barium Reagent Powder Pillows	1 pillow	100/pkg	12064-99
or BariVer® 4 Barium Reagent AccuVac® Ampuls	1 ampul	25/pkg	25130-25

Required Apparatus

Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Beaker, 50-mL	1	each	500-41H

Required Standards

Barium Standard Solution, 1000-mg/L Ba	100 mL	14611-42
Water, deionized	4 liters	272-56

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

DR/2400 Benzotriazole or Tolyltriazole

Method 8079

UV Photolysis Method*

Powder Pillows

Benzotriazole (1 to 16 mg/L)
Tolyltriazole (1 to 20 mg/L)

Scope and Application: For cooling or boiler water

* Adapted from Harp, D., *Proceedings 45th International Water Conference*, 299 (October 22–24, 1984)

Tips and Techniques

- Sample temperature should be 20–25 °C (68–78 °F).
- If sample contains nitrite or borax (sodium borate), adjust the pH to 4–6 with 1 N sulfuric acid (Cat. No. 1270-32).
- If the sample contains more than 500 mg/L hardness (as $CaCO_3$), add 10 drops of Rochelle Salt Solution (Cat. No. 1725-33) before adding reagent.
- Avoid fingerprints on the quartz surface of the lamp. Rinse the lamp and wipe with a soft, clean tissue between tests.

Powder Pillows — Method 8079

1. Touch

Hach Programs.

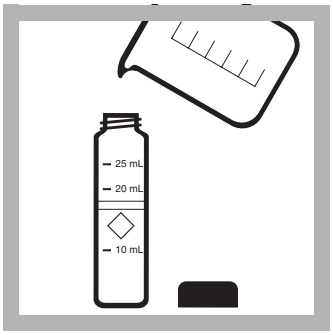
Select program

30 Benzotriazole

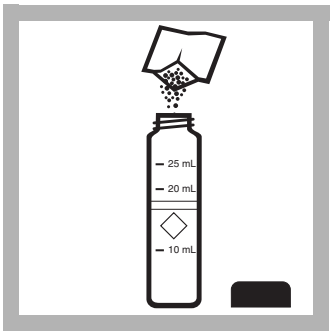
or

730 Tolyltriazole.

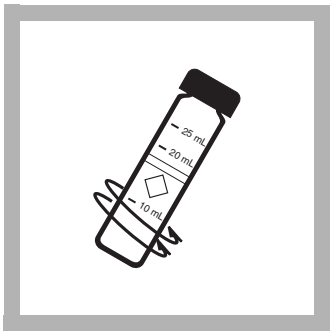
Touch **Start**.



2. Fill a round sample cell with 25 mL of sample.

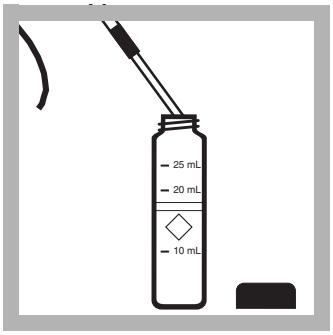


3. Add the contents of one Triazole Reagent Powder Pillow.



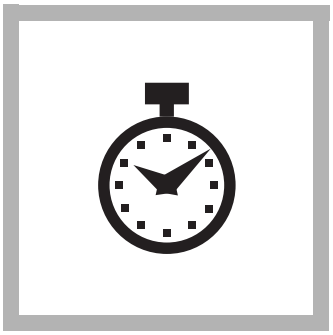
4. Swirl to dissolve completely.

5. Put on UV safety goggles.



6. Insert the ultraviolet lamp into the sample cell.

Turn on the UV lamp.

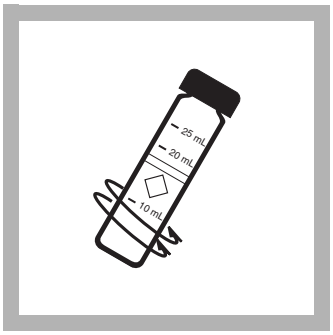


7. Touch the timer icon.

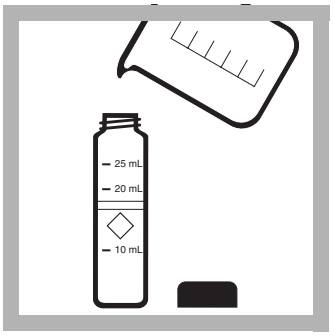
Touch **OK**.

A 5-minute reaction period will begin. Low results will occur if photolysis (lamp on) takes place for more or less than five minutes.

A yellow color will form if triazole is present.

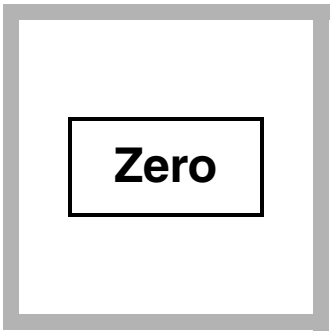


8. When the timer beeps, turn the lamp off. Remove the lamp from the cell (the prepared sample). Swirl the cell to mix thoroughly.



9. Fill another round sample cell with 25 mL of sample (the blank).

10. Place the blank into the cell holder.



11. Touch **Zero**.

The display will show:

0 mg/L Benzotr

or

0 mg/L Tolyltr

12. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L Benzotr or mg/L Tolyltr.

Interferences

Interfering Substance	Interference Levels and Treatments
Acrylates (as methyl acrylate)	Greater than 50 mg/L
Alum	Greater than 400 mg/L
Borate (as sodium tetraborate)	Greater than 4000 mg/L. Adjust the pH to 4–6 with 1 N sulfuric acid (Cat. No. 1270-32).
Chlorine (as Cl_2)	Greater than 20 mg/L

Interferences (continued)

Interfering Substance	Interference Levels and Treatments
Chromium (as chromate)	Greater than 12 mg/L
Copper	Greater than 10 mg/L
Hardness	Greater than 500 mg/L as $CaCO_3$. Add 10 drops of Rochelle Salt Solution (Cat. No. 1725-33) before adding reagent.
Iron	Greater than 20 mg/L
Lignosulfonates	Greater than 40 mg/L
Magnesium	Greater than 300 mg/L as $CaCO_3$
Molybdenum (as molybdate)	Greater than 200 mg/L
Nitrite	Greater than 4000 mg/L. Adjust the pH to 4–6 with 1 N sulfuric acid (Cat. No. 1270-32).
Phosphonates (AMP or HEDP)	Greater than 100 mg/L
Sulfate	Greater than 200 mg/L
Zinc	Greater than 80 mg/L
Strong oxidizing or reducing agents	Interfere at all levels

Sample Collection, Storage, and Preservation

The most reliable results are obtained when samples are analyzed as soon as possible after collection.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a 500-mg/L Benzotriazole Standard Solution.
5. Prepare three sample spikes. Fill three mixing cylinders with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page 32 for more information.

UV Lamp Check

To verify the ultraviolet lamp (normal life equals 5000 hours) is working properly, perform the following test:

1. Prepare a 5.0 mg/L benzotriazole standard solution by pipetting 10.0 mL of Benzotriazole Standard Solution, 500-mg/L benzotriazole, into a 1-liter volumetric flask. Dilute to volume.
2. Analyze according to the above procedure. If the result is significantly below 5 mg/L, replace the lamp.

Method Performance

Precision

Standard: 4 mg/L benzotriazole or tolyltriazole

Program	95% Confidence Limits of Distribution
30	3–5 mg/L benzotriazole
730	3–5 mg/L tolyltriazole

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
30	Entire range	0.010	1 mg/L benzotriazole
730	Entire range	0.010	1 mg/L tolyltriazole

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Benzotriazole or tolyltriazole, used in many applications as corrosion inhibitors for copper and copper alloys, are determined by a proprietary catalytic ultraviolet (UV) photolysis procedure requiring less than 10 minutes to perform. Test results are measured at 425 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Triazole Reagent Powder Pillows	1 pillow	100/pkg	21412-99

Required Apparatus

UV Safety Goggles	1	each	21134-00
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Select one based on available voltage:			
Lamp Kit, UV, with power supply, 115 VAC, 60 Hz	1	each	20828-00
Lamp Kit, UV, with power supply, 230 VAC, 50 Hz	1	each	20828-02

Required Standards

Benzotriazole Standard Solution, 500-mg/L	0.6 mL	100 mL	21413-42

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In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 8015 **Carmine Method***

Powder Pillows **(0.2 to 14.0 mg/L)**

Scope and Application: For water and wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

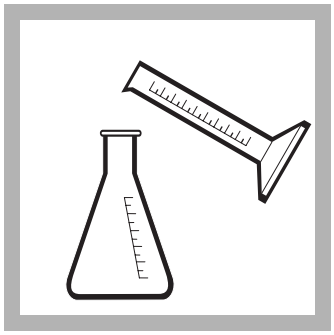
- All labware must be completely dry. Excess water will cause low results.
- Use the BoroVer® 3 Reagent with adequate ventilation. See *Reagent Preparation*, below.
- Do not cap the sample cells or the Erlenmeyer flasks at any time during sample preparation or reaction time. Samples may be capped immediately prior to placing in the instrument.
- Sulfuric acid may contain residual moisture; this will cause low results. It is advisable to ensure sulfuric acid suitability by running a known boron standard before running any unknown samples.

Powder Pillows Method 8015

1. Touch **Hach Programs**.

Select program **40 Boron**.

Touch **Start**.



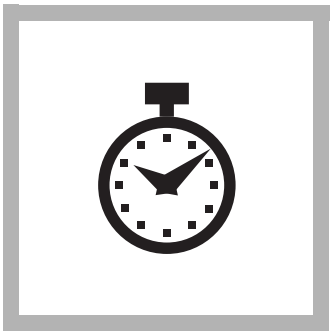
2. Using a 100-mL graduated cylinder, measure 75 mL of concentrated sulfuric acid. Pour the acid into a 250-mL Erlenmeyer flask.

3. Add the contents of one BoroVer 3 Reagent Powder Pillow to the flask. Swirl to mix. Allow up to five minutes for the powder to dissolve completely.

4. Accurately pipet 2.0 mL of deionized water into a 125-mL Erlenmeyer flask (the blank).

5. Accurately pipet 2.0 mL of sample into another 125-mL Erlenmeyer flask (the prepared sample).

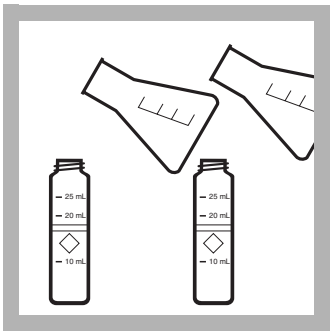
6. Using a 50-mL graduated cylinder, add 35 mL of the solution prepared in step 3 to each Erlenmeyer flask. Swirl to mix completely.



7. Touch the timer icon.

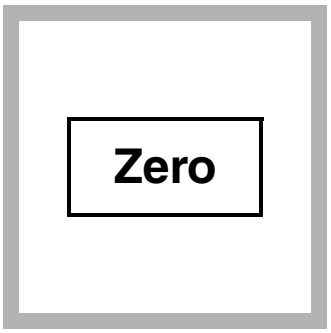
Touch **OK**.

A 25-minute reaction period will begin.



8. When the timer beeps, pour at least 10 mL from each flask into separate round sample cells. Cap the cells.

9. Place the blank into the cell holder.

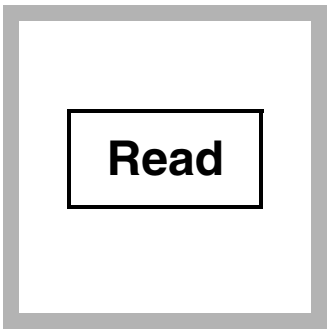


10. Touch **Zero**.

The display will show:

0.0 mg/L B

11. Place the prepared sample into the cell holder.



12. Touch **Read**.

Results will appear in mg/L B.

Sample Collection, Preservation, and Storage

Collect samples in clean polyethylene or polypropylene bottles, or alkali-resistant, boron-free glass.

Reagent Preparation

To prepare additional BoroVer 3/Sulfuric Acid Solution, mix one BoroVer 3 Reagent Powder Pillow per 75 mL of concentrated sulfuric acid, adding the powder pillows individually while stirring. Preparation of this solution generates gaseous HCl when the indicator pillow is added to the sulfuric acid. Use of a fume hood or other well-ventilated lab area is strongly advised. This solution is stable for up to 48 hours if stored in plastic containers. Do not store in Pyrex® or Kimex® glassware for longer than one hour; the solution may leach boron from these containers.

The BoroVer 3/Sulfuric Acid Solution is highly acidic. Neutralize to pH 6–9 and flush down the drain for disposal. For more information on waste management, see *Section 4*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the unspiked sample in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Boron Voluette® Ampule Standard, 250-mg/L B.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze 2.0 mL of each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Check the accuracy of the test using Boron Standard Solution, 10-mg/L as B. Prepare this solution as follows:

1. Using Class A glassware, pipet 10.00 mL of the Boron Voluette Ampule Standard, 250-mg/L B, into a 250-mL volumetric flask. Dilute to volume with deionized water. Swirl to mix.
2. To adjust the calibration curve using the reading obtained with the 10.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust: Off**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 10.0 mg/L B

Program	95% Confidence Limits of Distribution
40	9.5–10.5 mg/L B

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
0.010 mg/L	0.010	0.14 mg/L B
6 mg/L	0.010	0.15 mg/L B
13 mg/L	0.010	0.17 mg/L B

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Boron is determined by its reaction with carminic acid in the presence of sulfuric acid to produce a reddish to bluish color. The amount of color is directly proportional to the boron concentration. Test results are measured at 605 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
BoroVer® 3 Boron Reagent Powder Pillows	1 pillow	100/pkg	14170-99
Sulfuric Acid, ACS, concentrated	75 mL	2.5 liters	979-09
Water, deionized	2.0 mL	4 liters	272-56

Required Apparatus

Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 100-mL	1	each	508-42
Flask, Erlenmeyer, 125-mL	2	each	505-43
Flask, Erlenmeyer, 250-mL	1	each	505-46
Pipet, volumetric, 2.0-mL	2	each	14515-36
Sample cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Boron Standard Solution, 10-mL Voluette® Ampule, 250-mg/L B		16/pkg	14249-10

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Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 10061 **Azomethine-H Method***

Powder Pillows **LR (0.02 to 1.50 mg/L as B)**

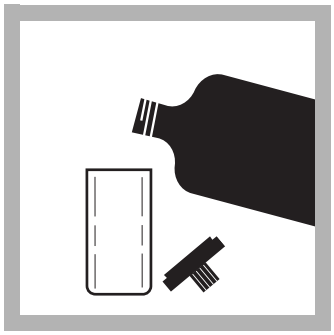
Scope and Application: For testing low levels of boron (boric acid or borates) in drinking water, cooling water, industrial process waters, or wastewaters

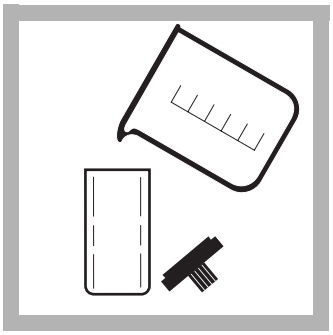
* Adapted from ISO Method 9390

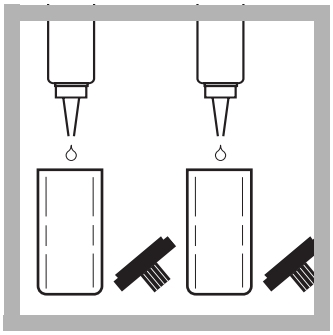
Tips and Techniques

- Sample temperature should be 22–24 °C (72–75 °F) for most accurate results. If outside this range, measure and record the sample temperature. See *Sample Temperature Compensation on page 4*.

Powder Pillows Method 10061







1. Touch **Hach Programs**.

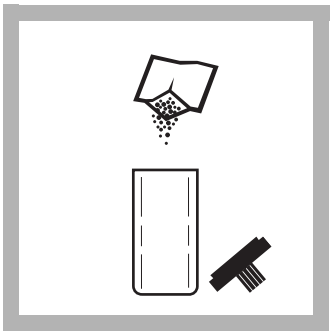
Select program **45 Boron LR**.

Touch **Start**.

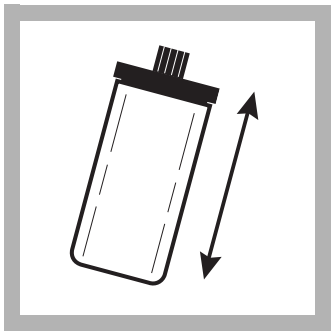
2. Fill a clean plastic sample cell to the 25-mL mark with ultra-pure water (the blank).

3. Fill a second clean plastic sample cell to the 25-mL mark with sample (the prepared sample).

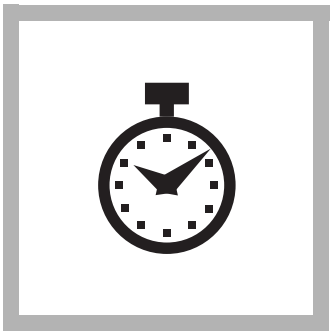
4. Add ten drops of EDTA Solution, 1 M, to each cell. Cap and invert each cell twice to mix.



5. Open one pillow BoroTrace™ 2 Reagent and add the contents to the prepared sample.



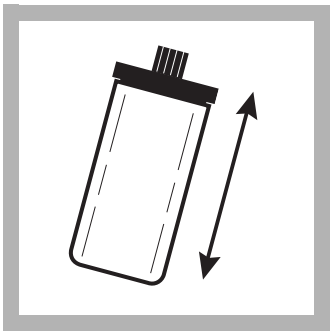
6. Cap the prepared sample and shake to dissolve the powder. Proceed immediately with steps 7 and 8.



7. Touch the timer icon.

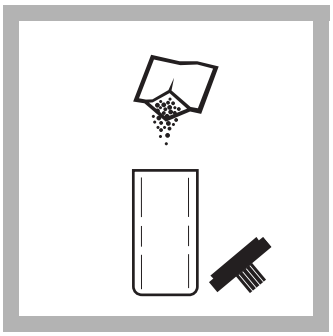
Touch **OK**.

A ten-minute reaction period will begin.

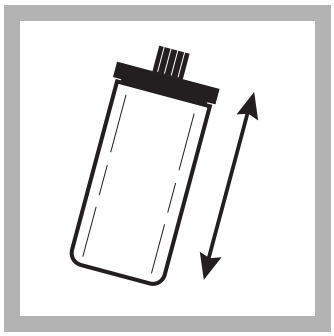


8. Continue shaking vigorously for 30 seconds, or until all powder is dissolved

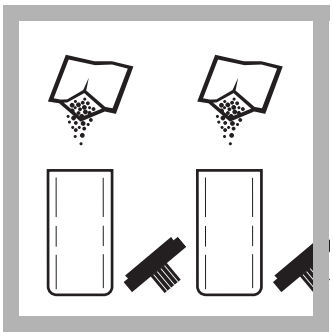
Let the cell sit capped for the remaining reaction period.



9. During the reaction period, add the contents of a second BoroTrace™ 2 Reagent Powder Pillow to the blank.



10. Cap the blank and shake vigorously until the powder is dissolved.

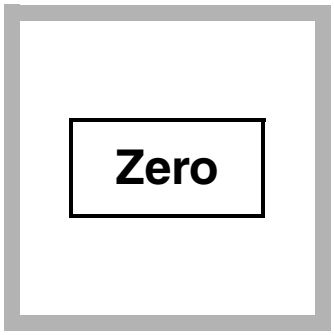


11. After the timer beeps, add the contents of one BoroTrace™ 3 Reagent Powder Pillow to each cell. Cap and shake to dissolve.

BoroTrace™ 3 Reagent "stops" the reaction.

12. Wipe the sample cells with tissue to remove fingerprints or other marks.

13. Place the blank into the cell holder.

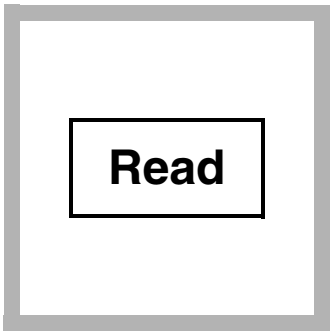


14. Touch **Zero**.

The display will show:

0.00 mg/L B

15. Place the prepared sample into the cell holder.



16. Touch **Read**.

Results will appear in mg/L B.

Cell Matching Procedure

1. Rinse and fill two cells with deionized water.
2. Wipe each cell with a soft cloth or tissue.
3. Using one of the cells, set the instrument absorbance at 410 nm to zero.
4. Read the absorbance of the other cell.
5. Cells that read within 0.002 absorbance are matched.

Interferences

The following have been tested for interference and found not to interfere up to the indicated levels (in mg/L):

Table 1 Interferences

Substance	Interference Level Tested
Aluminum ($^{3+}$)	10
Benzotriazole	20
Biocides:	
Carbamate-type	120
Isothiazolin-type	120
Quat-type	90
Thiocyanate-type	60
Calcium	1000 (as $CaCO_3$)
Chloride	2500
Copper ($^{2+}$)	20
Magnesium	1000 (as $CaCO_3$)
Manganese ($^{7+}$)	5
Molybdate (Mo^{6+})	60
Phosphonates, AMP	20
Phosphonates, HEDP	20
Polyacrylates	20 (as Acumer 1000, 1100)
Polymaleic Acid	40 (as Belcene 200)

Table 1 Interferences (continued)

Substance	Interference Level Tested
Silica	120
Sulfate	1800
Sulfite	40
Tolyltriazole	20
Zinc ($^{2+}$)	10

Table 2 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Alkalinity >500 mg/L (+ or -)	**1.** Adjust sample pH to between 5–7 using 1.0 N Sulfuric Acid Solution (Cat. No. 1270-32). **2.** Continue with step 4 of the analytic procedure.
Color (+)	**1.** Zero the instrument (0.00 mg/L B) using Ultra-Pure Water (Cat. No. 25946-49). **2.** Measure and record the apparent concentration, in mg/L B, due to the samples color. **3.** Subtract the apparent concentration from the result in step 16 of the test procedure.
Halogens (Bromine or Chlorine) all levels (+)	Halogen disinfectants in the sample can produce a red-color after the addition of BoroTrace™ 2 Reagent. To eliminate this interference: **1.** Add 1 pillow Dechlorinating Reagent (Cat. No. 14363-69) to 25 mL each of Ultra-Pure Water (Cat. No. 25946-49) and sample. **2.** Cap and shake to dissolve. **3.** Continue with step 4 of the test procedure.
Iron (Fe^{3+} or Fe^{2+}), above 8 mg/L (+)	High levels of iron in the sample can produce a red-color after the addition of BoroTrace™ 2 Reagent. To compensate, increase the amount of EDTA (Cat. No. 22419-26) from 10 drops to 15 drops to be added to each cell (Step 5). Alternatively, dilute the sample with Ultra-Pure Water and continue with Step 5 of the test procedure. Correct the results (Step 15) using the appropriate dilution factor.
Nitrites, all levels (+)	**1.** Add 0.1 gram scoop Sulfamic Acid (Cat. No. 2344-14) to 25 mL each Ultra-Pure Water and sample in plastic cells. **2.** Cap and shake to dissolve. **3.** Uncap and wait 5 minutes. **4.** Add 5 N Sodium Hydroxide Reagent (Cat. No. 2450-26) solution to each to adjust pH 5–8 using pH paper. **5.** Continue with step 4 of the test procedure.
Turbidity (+)	Filter the sample through a 3 µm membrane (Cat. No. 25940-25) prior to testing. Do not use a glass fiber filter.

Sample Collection, Preservation and Storage

Collect samples in clean polyethylene bottles. Do not use borate-based detergents or soaps to clean sample containers or labware used for this method. After use, thoroughly rinse all plastic containers with deionized water, allow to air dry, and keep covered.

Sample Temperature Compensation

The reaction chemistry is very dependent on the sample temperature. Hach calibrations are performed at 23 °C (73 °F). If the sample temperature is outside the range of 22–24 °C (72–75 °F), multiply the results, in mg/L, by the appropriate multiplier (*Table 3*).

Table 3 Sample Temperature Multipliers

Sample Temp.		Multiplier	Sample Temp.		Multiplier
°C	°F		°C	°F	
5	41	0.70	20	68	0.94
7	45	0.73	25	77	1.04
10	10	0.78	26	79	1.06
12	54	0.81	27	81	1.08
14	57	0.84	28	82	1.10
16	61	0.87	29	84	1.12
18	64	0.91	30	86	1.15

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare a 50.0-mg/L boron standard by pipetting 5.0 mL of a 1000-mg/L Boron Standard Solution into a 100-mL plastic volumetric flask. Dilute with deionized water, stopper and invert to mix.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 1.0-mg/L B standard as follows:

1. Using plastic pipet, transfer 4.0 mL of Boron Standard Solution, 250-mg/L as B, into a 1000-mL plastic volumetric flask. Dilute to volume with deionized water, stopper and invert to mix.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.

3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.30 mg/L B^{3+}

Program	95% Confidence Limits of Distribution
45	0.23–0.37 mg/L B

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.01 mg/L B

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Azomethine-H, a Schiff base, is formed by the condensation of an aminonaphthol with an aldehyde by the catalytic action of boron. Test results are measured at 410 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
BoroTrace™ Reagent Set			26669-00
Includes:			
EDTA Solution, 1 M	20 drops	50 mL SCDB	22419-26
BoroTrace™ #2 Reagent Powder Pillows	2	100/pkg	26666-69
BoroTrace™ #3 Reagent Powder Pillows	2	100/pkg	26667-99
Water, Ultra-Pure, Aldehyde-Free	25 mL	500 mL	25946-49

Required Apparatus

Cell, sample, 25-10 mL/1-cm	2	2/pkg	48643-02

Required Standards

Boron Standard Solution, 10-mL Voluette® Ampule, 250-mg/L B		16/pkg	14249-10
Boron Standard Solution, 1000-mg/L as B		100 mL	1914-42

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Method 8016 — DPD Method*

Powder Pillows or AccuVac® Ampuls — (0.05 to 4.50 mg/L)

Scope and Application: For testing bromine residuals (including hypobromite, hypobromous acid and bromamines) used as disinfectants in process waters, treated water, estuary water, and seawater

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- If the sample temporarily turns yellow after reagent addition, dilute a fresh sample and repeat the test. A slight loss of bromine may occur because of the dilution. Multiply the result by the dilution factor. See *Section 2.7 Sample Dilution* on page *21*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows — Method 8016

1. Touch

Hach Programs.

Select program

50 Bromine.

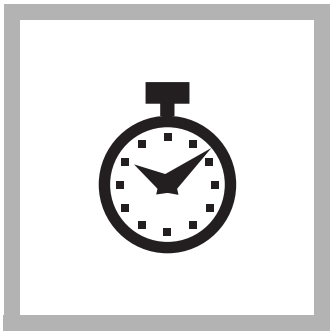
Touch **Start**.

2. Fill a sample cell with 10 mL of sample.

3. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell. (This is the prepared sample.)

Swirl to mix.

A pink color will develop if bromine is present.



4. Touch the timer icon.

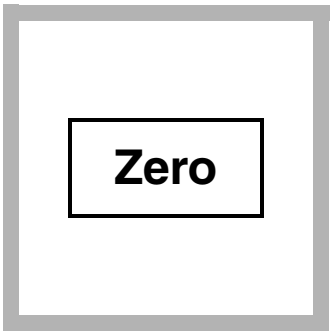
Touch **OK**.

A three-minute reaction period will begin.

Perform *steps 5–7* during the reaction period.

5. Fill a second sample cell with 10 mL of sample. (This is the blank.)

6. Wipe the blank and place it into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L Br_2

8. Within three minutes after the timer beeps, wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L Br_2.

AccuVac® Ampul — Method 8016

1. Touch

Hach Programs.

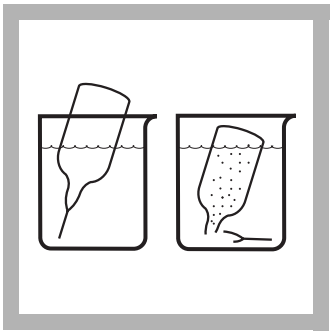
Select program

55 Bromine, AV.

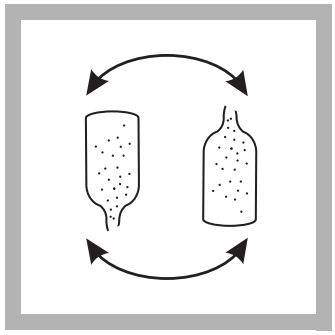
Touch **Start**.

2. Fill a sample cell with 10 mL of sample. (This is the blank.)

Note: *Collect at least 40 mL of sample in a 50-mL beaker.*

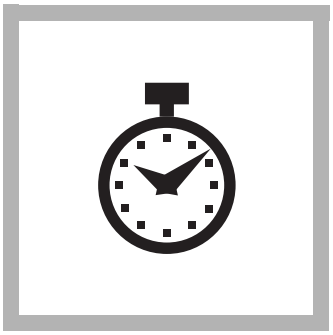


3. Fill a DPD Total Chlorine Reagent AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampules several times to mix. Wipe off liquid or fingerprints.

A pink color will develop if bromine is present.



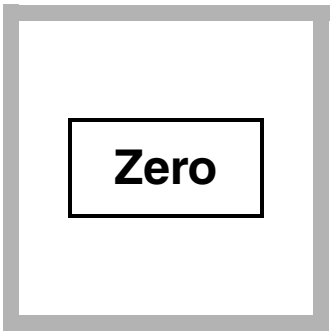
5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.

Perform *steps 6–7* during the reaction period.

6. Wipe the blank and place it into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L Br_2

8. Within three minutes after the timer beeps, wipe the ampul and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L Br_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide (Cat. No. 1045-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Chlorine	Interferes at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as $CaCO_3$
Iodine	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide (30-g/L) (Cat. No. 343-32) to a 25-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops Sodium Arsenite* (5-g/L) (Cat. No. 1047-32) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. Subtract the result from this test from the original analysis to obtain the correct bromine concentration.
Monochloramine	Interferes at all levels
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7. See *Section 3.3 Interferences* on page *41*.

* Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004).

Sample Collection, Storage, and Preservation

Collect samples in clean, dry glass containers. If sampling from a tap, allow the water to flow at least 5 minutes to ensure a representative sample. Avoid excessive agitation and exposure to sunlight when sampling. Allow several volumes of water to overflow the container and cap the container so there is no headspace above the sample. If sampling with a DR cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Proceed with the analysis immediately.

Method Performance

Precision

Standard: 2.41 mg/L Br_2

Program	95% Confidence Limits of Distribution
50	2.36–2.45 mg/L Br_2
55	2.32–2.50 mg/L Br_2

See *Section 3.4.3 Precision* on page *44 Precision* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
50	Entire range	0.010	0.05 mg/L Br_2
55	Entire range	0.010	0.05 mg/L Br_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Bromine residuals reacts with DPD (N,N-diethyl-p-phenylenediamine) to form a pink color which is proportional to the total bromine concentration. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
DPD Total Chlorine Reagent Powder Pillows	1 pillow	100/pkg	21056-69
or DPD Total Chlorine Reagent AccuVac® Ampuls	1 ampul	25/pkg	25030-25

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cells, 10 mL, w/cap	2	6/pkg	24276-06

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Telephone: (970) 669-3050
FAX: (970) 669-2932

Cadmium

Cadion Method

(0.02 to 0.30 mg/L Cd)

UniCell™ Vials

Scope and Application: For wastewater process control

Tips and Techniques

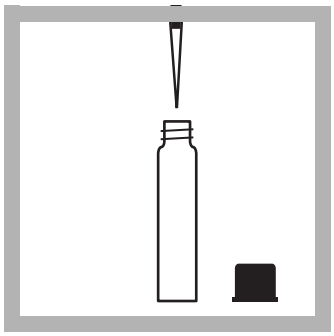
- Adjust the pH to between 3–6 with 5.0 N Sodium Hydroxide Standard Solution before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a wet towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check* on page *3*.
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure that the temperature of the sample and the reagents is between 15–25 °C.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

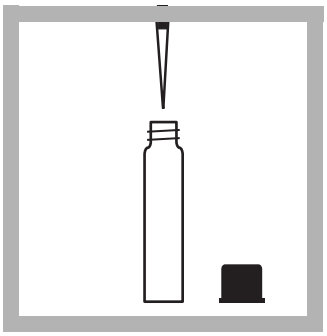
1. Touch **Hach Programs**.

Select program **815 Cadmium, HCT 154**.

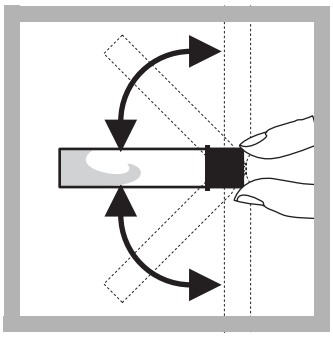
Touch **Start**.



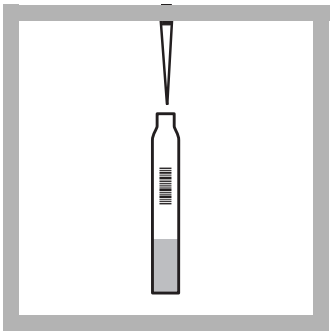
2. Pipet 10.0 mL of sample into the reaction tube (**red** cap).



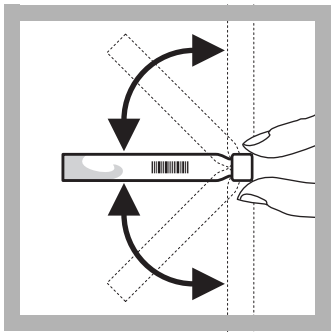
3. Pipet 1.0 mL of Complexing Solution A (HCT 154 A) into the reaction tube.



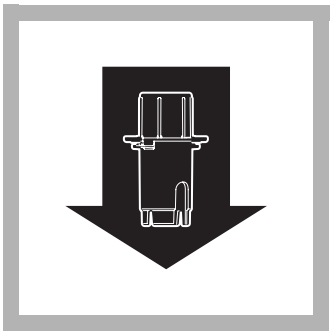
4. Close the reaction tube and invert several times to mix.



5. Pipet 0.5 mL of Stabilizer B (HCT 154 B) into a sample vial.



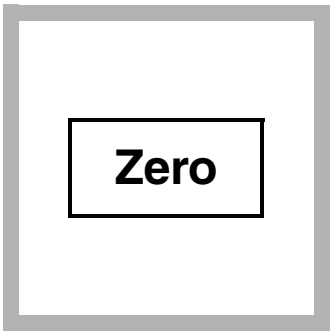
6. Close the sample vial and invert several times to mix.



7. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Wipe the sample vial and place it into the cell adapter.

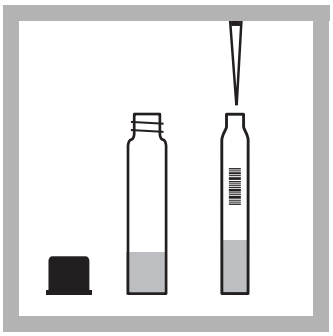


8. Touch **Zero**.

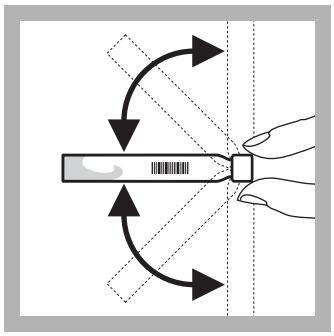
The display will show:

0.00 mg/L Free Cd

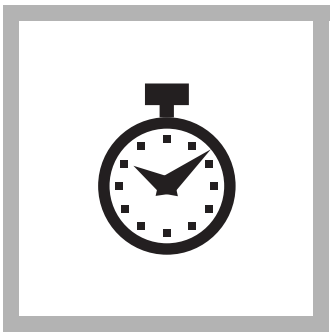
Underrange



9. Pipet 5.0 mL of sample from the reaction tube into the sample vial.



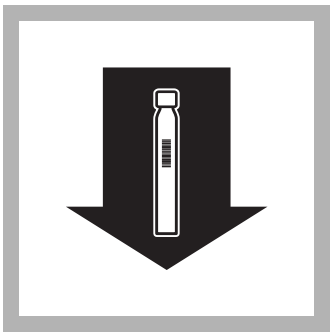
10. Close the sample vial and invert several times to mix.



11. Touch the timer icon.

Touch **OK**.

A 30-second reaction period will begin.



12. Wipe the sample vial and place it into the celladapter.

Touch **Read**.

Results will appear in mg/L Cd.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Ca^{2+}, Mg^{2+}	50 mg/L
Ag^{+}, Au^{+}, Cr^{6+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}	25 mg/L
Mn^{2+}	2 mg/L

Total cadmium, including undissolved cadmium and complexed cadmium can only be determined after digesting with the Metal Prep Set, HCT 200.

Note: *The total cadmium measuring range is 0.02–0.36 mg/L.*

Sample Collection, Storage, and Preservation

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3 and 6 with 5.0 N Sodium Hydroxide standard solution. Water samples which are free from complexing agents and organic compounds can be analyzed directly. Other water samples have to be digested with the Metal Prep Set in order to bring undissolved or complexed cadmium compounds into solution.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
2. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
3. Prepare three sample spikes. Fill three mixing cylinders with 250 mL of sample. Use a pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of 100-mg/L Cd standard, respectively, to each sample and mix thoroughly.
4. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
5. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 0.20-mg/L Cd standard solution by pipetting 0.20 mL of 100-mg/L Cd standard into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the cadmium procedure as described.

To adjust the calibration curve using the reading obtained with the 0.20-mg/L Cd standard solution:

1. Touch **Options** on the current program menu. Touch **Standard Adjust**.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.20 mg/L Free Cd

Program	95% Confidence Limits of Distribution
815	0.13–0.27 mg/L Free Cd

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
815	Entire range	0.010	0.015 mg/L Free Cd

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Cadmium ions react with cadion to form a red complex. This complex is then broken down and the resulting reduction in color is measured. Measurements are taken at 552 nm.

Required Reagents

Description	Unit	Cat. No.
Cadmium - Cd, UniCell™ HCT 154	24/pkg	HCT 154

Optional Reagents

Cadmium Standard Solution, 100-mg/L as Cd	100 mL	14024-42
Metal Prep Set	50 digestions	HCT 200
Nitric Acid, concentrated	500 mL	152-49
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	2450-26

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Flask, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 250-mL	each	20886-46
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	400/pkg	27950-00
pH Paper	100/pkg	26013-00

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Chloramine (Mono)

Method 10172

Indophenol Method*
HR (0.1 to 10.0 mg/L Cl_2)

Scope and Application: Chlorinated wastewater.

* Patent Pending

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Test "N Tube — Method 10172

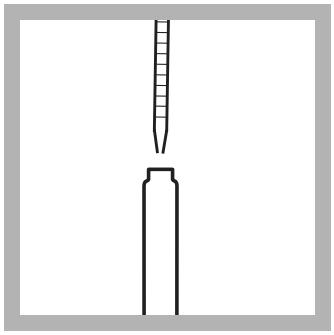
1. Touch

Hach Programs.

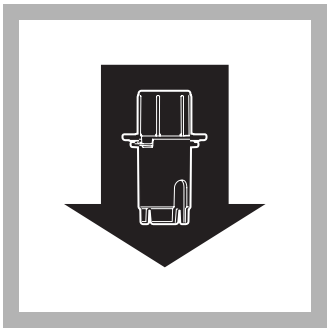
Select program

67 Monochlor. HR TNT.

Touch **Start**.

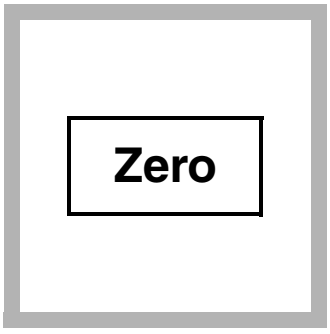


2. Remove the cap from one HR Monochloramine Diluent vial. Use a glass pipet to add 2.0 mL sample to the vial. Re-cap and invert several times to mix.



3. Install the 16-mm cell adapter. Wipe the vial and place it into the adapter.

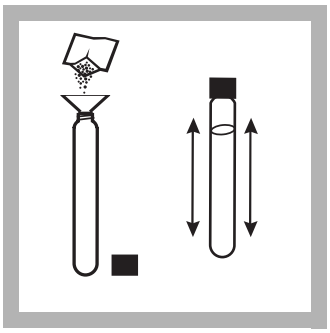
Note: *See Section 2.6 Sample Cell Adapters For adapter installation details.*



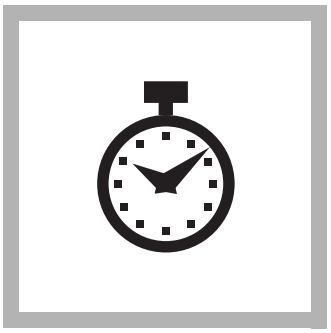
4. Touch **Zero**.

The display will show:

0.0 mg/L Cl_2



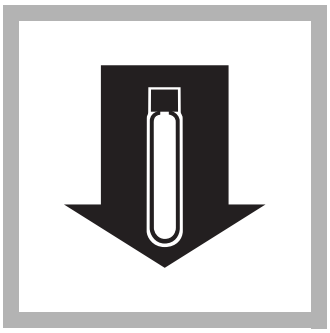
5. Remove the vial from the adapter. Using a micro-funnel, add the contents of one Monochlor F pillow to the sample. Cap and shake the cell about 20 seconds to dissolve.



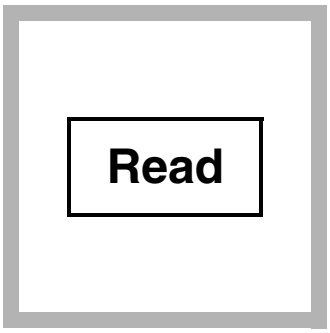
6. Touch the timer icon.

Touch **OK**.

A 5-minute reaction period will begin.



7. After the color has developed fully, place the vial into the cell adapter.



8. Touch **Read**.

Results will appear in mg/L Cl_2.

Interferences

The following have been tested for interference and found *not* to interfere up to the indicated levels:

Table 1 Non-interfering Substances

Substance	Maximum Level Tested
Alanine	1 mg/L N
Aluminum	10 mg/L
Bromide	100 mg/L Br^-
Bromine	15 mg/L Br_2
Calcium	1000 mg/L as $CaCO_3$
Chloride	18,000 mg/L
Chlorine Dioxide	5 mg/L ClO_2
Chromium (III)	5 mg/L
Copper	10 mg/L
Cyanide	10 mg/L CN^-
Dichloramine	10 mg/L as Cl_2
Fluoride	5 mg/L
Free Chlorine	10 mg/L Cl_2
Glycine	1 mg/L N
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Magnesium	1000 mg/L as $CaCO_3$
Manganese (VII)	10 mg/L
Lead	10 mg/L

Table 1 Non-interfering Substances (continued)

Substance	Maximum Level Tested
Nitrate	100 mg/L N
Nitrite	50 mg/L N
Phosphate	100 mg/L PO_4
Silica	100 mg/L SiO_2
Sulfate	2600 mg/L
Sulfite	50 mg/L SO_3^{2-}
Tyrosine	1 mg/L N
Urea	10 mg/L N
Zinc	5 mg/L

Table 2 Interfering Substances and Suggested Treatments

Interfering Substance and its effect		Interference Level	Recommended Treatment
Ozone	–	Above 1 mg/L	Usually doesn't coexist with monochloramine.
Sulfide	+	Turns a "rust" color if present.	Usually doesn't coexist with monochloramine.
Thiocyanate	–	Above 0.5 mg/L	This method will tolerate up to 2 mg/L.

Sampling and Storage

Analyze samples for monochloramine immediately after collection. Rinse the sample container several times with sample, letting the container overflow each time. If sampling from a tap, let the water flow for at least 5 minutes. Then cap the container so that there is no head space (air) above the sample.

Accuracy Check

To check test accuracy, prepare the following 4.5-mg/L (as Cl_2) monochloramine standard immediately before use.

1. Add the contents of one Buffer Powder Pillow, pH 8.3 to about 50-mL of organic-free water in a clean 100-mL Class A volumetric flask. Swirl to dissolve the powder.
2. Using a Class A volumetric pipet, transfer 2.00 mL of Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N into the flask.
3. Dilute to volume with organic-free water, cap and mix thoroughly. This is a 2.00-mg/L buffered ammonia standard.
4. Pipet 50.00 mL of the buffered ammonia standard into a clean 100-mL beaker. Add a stir bar.
5. Obtain a recent lot of Chlorine Solution Ampules, 50–70 mg/L, and note the actual free chlorine concentration for this lot.
6. Calculate the amount of Chlorine Solution to be added to the ammonia standard using the following equation:

$$\text{mL chlorine solution required} = \frac{455}{\text{free chlorine concentration}}$$

7. Open an ampule and use a glass Mohr pipet to add the calculated amount of Chlorine Solution slowly to the ammonia standard, while mixing at medium speed on a stir-plate.
8. Allow the monochloramine solution to mix for 1 minute after all Chlorine Solution is added.
9. Quantitatively transfer the monochloramine solution to a clean 100-mL Class A volumetric flask. Dilute to the mark with organic-free water, cap, and mix thoroughly. This is a nominal 4.5-mg/L (as Cl_2) monochloramine standard.
10. Use this standard within 1 hour of preparation. Analyze according to the Low Range Monochloramine procedure described above.
11. To adjust the calibration curve using the reading obtained with the 4.5-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
12. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision
Standard: 5.0 mg/L Cl_2

Program	95% Confidence Limits of Distribution
67	4.8–5.2 mg/L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.1 mg/L Cl_2*

* Use the LR Chloramine (Mono) test for concentrations below 4.5 mg/L Cl_2.

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The sample is first diluted in a Test 'N Tube. In the presence of a cyanoferrate catalyst, monochloramine (NH_2Cl) in the sample reacts with a substituted phenol to form an intermediate monoimine compound. The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample. Test results are measured at 655 nm.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet (MSDS) for information specific to the reagent used.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
HR Monochloramine Test 'N Tubes, 50 tests			28051-45
Includes:			
(50) HR Monochloramine Diluent Vials			*
Funnel, micro	1	each	25843-35
Monochlor F Reagent Pillows	1	50/pkg	28022-66

Required Apparatus

Adapter, 16-mm Cell		each	59457-00
Pipet, Mohr, glass, 2.00-mL	1	each	20936-36
Test Tube Rack	1	each	18641-00

Optional Reagents

Organic-Free Water		500-mL	26415-49
Buffer Powder Pillows		pk/25	898-68
Chlorine Solution Voluette® Ampule		pk/16	14268-10
Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N		500-mL	24065-49

Optional Apparatus

Beaker, 100-mL		each	500-42
Clippers, for medium powder pillows		each	968-00
Flask, Volumetric, Class A, 100-mL		each	14574-42
Pipet, Mohr, Glass, 10-mL		each	20934-38
Pipet, Volumetric, Class A, 2.00-mL		each	14515-36
Pipet, Volumetric, Class A, 50.00-mL		each	14515-41
Stir Bar, Octagonal		each	20953-52
Stirrer, Magnetic		each	23436-00
Clippers (shears)		each	23694-00

* This item cannot be purchased separately.

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3/04 1ed

Chloramine (Mono)

Method 10171

Indophenol Method*
LR (0.04–4.50 mg/L Cl_2)

Scope and Application: Chloraminated drinking water and chlorinated wastewater

* Patent pending

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Install the Multi-pathlength Cell into the adapter oriented for the short (1-cm) pathlength (the long pathlength is perpendicular to the front of the instrument).

DR/2400 Multi-Path Cell — Method 10171

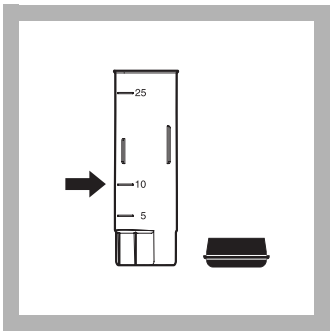
1. Touch

Hach Programs.

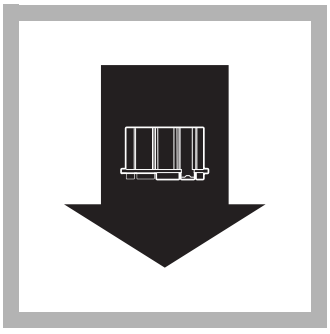
Select program

66 Monochlor. LR.

Touch **Start**.

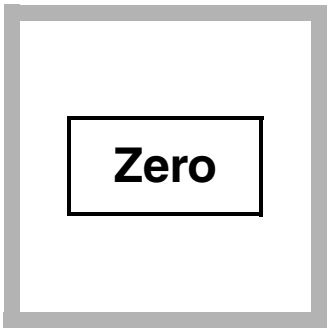


2. Fill the multi-pathlength cell to the 10-mL line with sample.



3. Install the adapter. Wipe the cell. Place the cell into the cell adapter and orient it for the short pathlength.

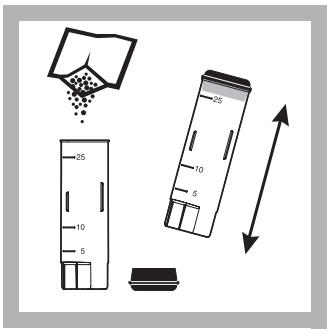
Note: *See Section 2.6.1 Installing Sample Cell Adapters in the Instrument Manual for adapter installation details.*



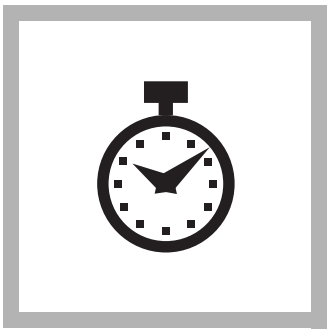
4. Touch **Zero**.

The display will show:

0.00 mg/L Cl_2



5. Remove the cell from theadapter and add the contents of one pillow Monochlor-F to the sample. Cap and shake the cell about 20 seconds to dissolve.



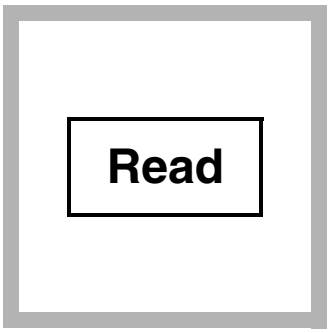
6. Touch the timer icon.

Touch **OK**.

A 5-minute reaction period will begin.

Note: *Samples colder than 18 °C will require additional time. See Table 3 on page 3.*

7. After the color has developed fully, wipe the cell and place it into the adapter.



8. Touch **Read**.

Results will appear in mg/L Cl_2.

Interferences

The following have been tested for interference and found *not* to interfere up to the indicated levels:

Table 1

Substance	Maximum Level Tested
Alanine	1 mg/L N
Aluminum	10 mg/L
Bromide	100 mg/L Br^-
Bromine	15 mg/L Br_2
Calcium	1000 mg/L as $CaCO_3$
Chloride	18,000 mg/L
Chlorine Dioxide	5 mg/L ClO_2
Chromium (III)	5 mg/L
Copper	10 mg/L
Cyanide	10 mg/L CN^-
Dichloramine	10 mg/L as Cl_2
Fluoride	5 mg/L
Free Chlorine	10 mg/L Cl_2
Glycine	1 mg/L N
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Lead	10 mg/L
Nitrate	100 mg/L N
Nitrite	50 mg/L N

Table 1 (continued)

Substance	Maximum Level Tested
Phosphate	100 mg/L PO_4
Silica	100 mg/L SiO_2
Sulfate	2600 mg/L
Sulfite	50 mg/L SO_3^{2-}
Tyrosine	1 mg/L N
Urea	10 mg/L N
Zinc	5 mg/L

Table 2

Interfering Substance and its effect		Interference Level	Recommended Treatment
Magnesium	+	Above 400 mg/L $CaCO_3$	Add 5 drops Rochelle Salt Solution prior to testing. OR: use the high range (HR) test.
Manganese (+7)	–	Above 3 mg/L	Use the HR test; it will tolerate up to 10 mg/L.
Ozone	–	Above 1 mg/L	Usually doesn't coexist with monochloramine.
Sulfide	+	Turns a "rust" color if present.	Usually doesn't coexist with monochloramine.
Thiocyanate	–	Above 0.5 mg/L	Use the HR test; it will tolerate up to 2 mg/L.

Table 3

Sample Temperature		Minutes
° C	° F	
5	40	10
7	42	9
9	48	8
10	50	8
12	54	7
14	58	7
16	61	6
18	68	4
20	73	3
23	75	2.5
25	77	2
>25	>77	2

Sampling and Storage

Analyze samples for monochloramine immediately after collection. Rinse the sample container several times with sample, letting the container overflow each time. If sampling from a tap, let the water flow for at least 5 minutes. Then cap the container so that there is no head space (air) above the sample.

Accuracy Check

To check test accuracy, prepare the following 4.5-mg/L (as Cl_2) monochloramine standard fresh before use.

1. Add the contents of one Buffer Powder Pillow, pH 8.3, to about 50-mL of organic-free water in a clean 100-mL Class A volumetric flask. Swirl to dissolve the powder.
2. Using a Class A volumetric pipet, transfer 2.00 mL of Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N into the flask.
3. Dilute to volume with organic-free water, cap, and mix thoroughly. This is a 2.00-mg/L buffered ammonia standard.
4. Pipet 50.00 mL of the buffered ammonia standard into a clean 100-mL beaker. Add a stir bar.
5. Obtain a recent lot of Chlorine Solution Ampules, 50–70 mg/L, and note the actual free chlorine concentration for this lot.
6. Calculate the amount of Chlorine Solution to be added to the ammonia standard using the following equation:

$$\text{mL chlorine solution required} = \frac{455}{\text{free chlorine concentration}}$$

7. Open an ampule and, using a glass Mohr pipet, add the calculated amount of Chlorine Solution slowly to the ammonia standard, while mixing at medium speed on a stir-plate.
8. Allow the monochloramine solution to mix for 1 minute after all Chlorine Solution is added.
9. Quantitatively transfer the monochloramine solution to a clean 100-mL Class A volumetric flask. Dilute to the mark with organic-free water, cap, and mix thoroughly. This is a nominal 4.5-mg/L (as Cl_2) monochloramine standard.
10. Use this standard within 1 hour of preparation. Analyze according to the High Range Monochloramine procedure described above.
11. To adjust the calibration curve using the reading obtained with the 4.5-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
12. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision

Standard: 2.52 mg/L Cl_2

Program	95% Confidence Limits of Distribution
66	2.41–2.63 mg/L Cl_2

See *Section 3.4.3 Precision* on page *42* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.04 mg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *43* for more information.

Summary of Method

In the presence of a cyanoferrate catalyst, monochloramine (NH_2Cl) in the sample reacts with a substituted phenol to form an intermediate monoimine compound. The intermediate couples with excess substituted phenol to form a green-colored indophenol, which is proportional to the amount of monochloramine present in the sample. Test results are measured at 655 nm.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet (MSDS) for information specific to the reagent used.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Monochlor F Reagent Pillows	1	50/pkg	28022-46

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cell, sample, multi-pathlength	2	6/pkg	59405-06
Clippers	1	each	23694-00

Optional Reagents

Buffer Powder Pillows		pk/25	898-68
Chlorine Solution Voluette® Ampule		pk/16	14268-10
Nitrogen, Ammonia Standard Solution, 100-mg/L as NH_3–N		500-mL	24065-49
Organic-Free Water		500-mL	26415-49
Rochelle Salt Solution		29-mL DB	1725-33

Optional Apparatus

Beaker, 100-mL		each	500-42
Flask, Volumetric, Class A, 100-mL		each	14574-42
Pipet, Mohr, Glass, 10-mL		each	20934-38
Pipet, Volumetric, Class A, 2.00-mL		each	14515-36
Pipet, Volumetric, Class A, 50.00-mL		each	14515-41
Stir Bar, Octagonal		each	20953-52
Stirrer, Magnetic		each	23436-00

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Method 8113

Mercuric Thiocyanate Method
(0.1 to 25.0 mg/L Cl^-)

Scope and Application: For water and wastewater

Tips and Techniques

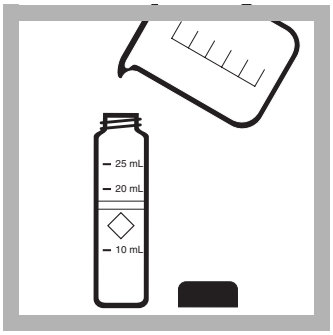
- Filter turbid samples with moderately rapid filter paper (Cat. No. 692-57) and a funnel (Cat. No. 1083-68) before analysis.
- Both the sample and the blank will contain mercury (D009) at a concentration regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. See *Waste Management and Safety* on page *55* for more information on proper disposal of these materials.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Method 8113

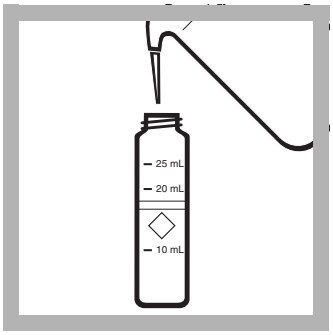
1. Touch **Hach Programs**.

Select program **70 Chloride**.

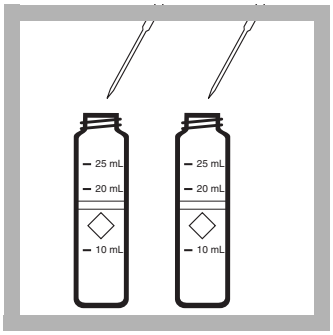
Touch **Start**.



2. Fill a round sample cell with 25 mL of sample. (This is the prepared sample.)

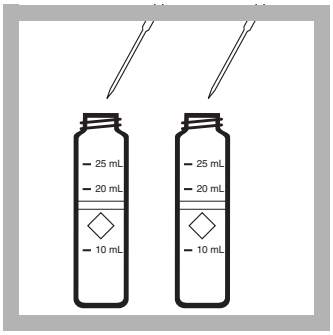


3. Fill another sample cell with 25 mL of deionized water. (This is the blank.)



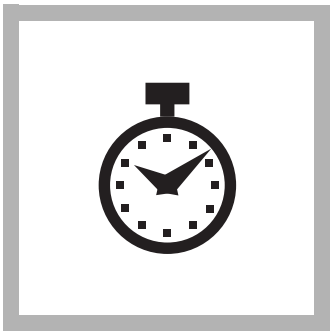
4. Pipet 2.0 mL of Mercuric Thiocyanate Solution into each sample cell.

5. Swirl to mix.



6. Pipet 1.0 mL of Ferric Ion Solution into each sample cell.

7. Swirl to mix. An orange color will develop if chloride is present.

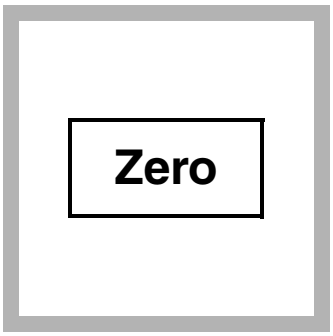


8. Touch the timer icon.

Touch **OK**.

A two-minute reaction time will begin.

9. Within five minutes after the timer beeps, wipe the blank and place it into the cell holder.

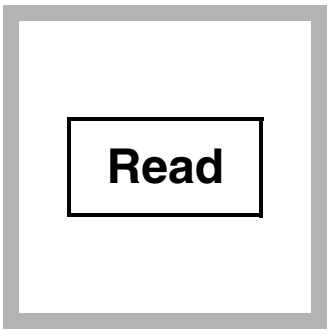


10. Touch **Zero**.

The display will show:

0.0 mg/L Cl⁻

11. Wipe the prepared sample and place it into the cell holder.



12. Touch **Read**.

Results will appear in mg/L Cl^-.

Interferences

Interfering Substance	Interference Levels and Treatments
Extreme pH	Should be about pH 2 after adding reagents. If the sample is strongly acidic or alkaline, adjust a portion of sample before testing to a pH of about 7. Use either 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-26) or a 1:5 dilution of perchloric acid (Cat. No. 757-65). **Use pH paper** (Cat. No. 391-33), as most pH electrodes will contaminate the sample with chloride.

Sample Collection, Storage, and Preservation

Collect samples in glass or plastic containers. Samples can be stored for at least 28 days at room temperature.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of 1000-mg/L Chloride Standard Solution, respectively, to three 25-mL samples and mix each thoroughly.
5. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 20.0-mg/L chloride standard solution as follows:

1. Using Class A glassware, pipet 10.00 mL of Chloride Standard Solution, 1000-mg/L, into a 500-mL volumetric flask.
2. Dilute to the mark with deionized water. Perform the chloride procedure as described above.
3. To adjust the calibration curve using the reading obtained with the 20.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 20.0 mg/L Cl^-

Program	95% Confidence Limits
70	18.3–21.8 mg/L Cl^-

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
1.0 mg/L	0.010	0.11 mg/L Cl^-
20.0 mg/L	0.010	45 mg/L Cl^-

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chloride in the sample reacts with mercuric thiocyanate to form mercuric chloride and liberate thiocyanate ion. Thiocyanate ions react with the ferric ions to form an orange ferric thiocyanate complex. The amount of this complex is proportional to the chloride concentration. Test results are measured at 455 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Chloride Reagent Set (50 Tests)*			23198-00
Includes:			
(1) Ferric Ion Solution	2 mL	100 mL	22122-42
(1) Mercuric Thiocyanate Solution	4 mL	200 mL	22121-29
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Sample cells, 10-20-25 mL, with cap	2	6/pkg	24019-06
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	1	50/pkg	21997-96

Required Standards

Chloride Standard Solution, 1000-mg/L Cl^-		500 mL	183-49
Chloride Standard Solution, 2-mL Voluette® Ampule, 12,500-mg/L Cl^-		20/pkg	14250-20

* 50 tests equals 25 samples and 25 blanks.

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Chlorine Dioxide

Method 8138

Direct Reading Method

HR (5 to 1000 mg/L)

Scope and Application: For water and wastewater

Tips and Techniques

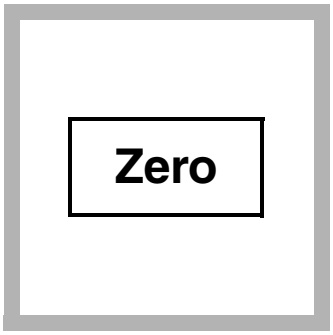
- Analyze samples immediately because chlorine dioxide is unstable and volatile. See *Interferences*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Method 8138

1. Touch **Hach Programs**.
Select program **75 Chlor Diox HR**.
Touch **Start**.

2. Fill a round sample cell to the 10-mL mark with deionized water. (This is the blank.)

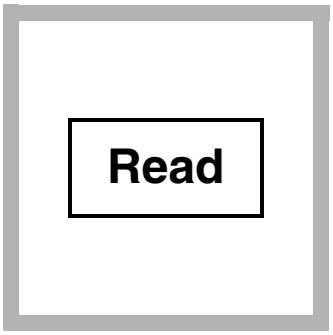
3. Wipe the blank and place it into the cell holder.



4. Touch **Zero**.
The display will show:
0 mg/L ClO_2

5. Fill another sample cell to the 10-mL mark with sample. (This is the prepared sample.)

6. Wipe the prepared sample and place it into the cell holder.



7. Touch **Read**.
Results will appear in mg/L ClO_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide (Cat. No. 1045-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions).*
Alkalinity	Greater than 250 mg/L $CaCO_3$. Color may not develop fully or may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for the volume addition (see *Section 3.1.3 Correcting for Volume Additions*).
Bromine, Br_2	Interferes at all levels.
Chlorine, Cl_2	May interfere at levels greater than 6 mg/L. Additional glycine may be able to compensate for this interference.
Chloramines, organic	May interfere.
Flocculating agents	High levels of most flocculating agents can be tolerated. This tolerance is decreased if chlorine is present. See the information about metals in this table. In the presence of 0.6 mg/L Cl_2, $Al(SO_4)_3$ (< 500 mg/L) and $FeCl_2$ (<200 mg/L) may be tolerated.
Hardness	No effect at less than 1,000 mg/L as $CaCO_3$.
Iodine, I_2	Interferes at all levels.
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	Oxidized manganese interferes at all levels. Oxidized chromium interferes at levels greater than 2 mg/L. To remove the interferences: **1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide (30 g/L) (Cat. No. 343-32) to a 25-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops Sodium Arsenite* (5 g/L) (Cat. No. 1047-32) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. Subtract the result of this test from the original analysis to obtain the correct chlorine dioxide concentration.
Metals	Various metals may interfere by combining with the glycine needed to remove the chlorine interference. Metal interference is limited except when chlorine is present. In the presence of 0.6 mg/L Cl_2, both copper (>10 mg/L) and nickel (>50 mg/L) interfere. Other metals may also interfere, depending on their ability to prevent glycine from reacting with any Cl_2 in the sample. It may be necessary to add more glycine to overcome this interference.
Monochloramine	Causes a gradual drift to higher readings. When read within 1 minute after reagent addition, 3 mg/L monochloramine causes less than a 0.1 mg/L ClO_2 increase in the reading.
Ozone	Interferes at levels greater than 1.5 mg/L.
Peroxides	May interfere.
Extreme sample pH	Adjust to pH 6–7. See *Section 3.3 Interferences.*
Highly buffered samples	Adjust to pH 6–7. See *Section 3.3 Interferences.*

* Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See *Section 4* for further information on proper disposal of these materials.

Sample Collection, Storage, and Preservation

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide

demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine dioxide analysis immediately.

Accuracy Check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 20th ed., under the headings "Stock chlorine dioxide solution" and "Standard chlorine dioxide solution" (pp 4–74 and 4–75). Prepare a 500-mg/L chlorine dioxide standard.

Method Performance

Precision

Standard: 500 mg/L ClO_2

Program	95% Confidence Limits of Distribution
75	499–501 mg/L ClO_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	5 mg/L ClO_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chlorine dioxide, a yellow gas, can be measured directly in a water solution. Test results are measured at 445 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No
Water, deionized	10 mL	4 liters	272-56

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

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★Method 10126 — DPD Method*

Powder Pillows and AccuVac® Ampuls — (0.04 to 5.00 mg/L)

Scope and Application: For water and wastewater. USEPA accepted for reporting for drinking water analysis.**

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Procedure is equivalent to *Standard Methods, 18 ed., 4500 ClO_2 D*.

Tips and Techniques

- Analyze samples immediately because chlorine dioxide is unstable and volatile. See *Sample Collection, Storage, and Preservation*.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using chlorine-free deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank.*
- After adding the DPD Free Chlorine Powder Pillow to the sample, a pink color will develop if chlorine dioxide is present.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- If the chlorine dioxide concentration in the sample exceeds the upper limit of the test, the color may fade or the sample may turn yellow. Dilute the sample with high quality water that is chlorine demand-free, and repeat the test. Some loss of chlorine dioxide may occur. Multiply the result by the appropriate dilution factor.

Powder Pillows — Method 10126

1. Touch **Hach Programs**.

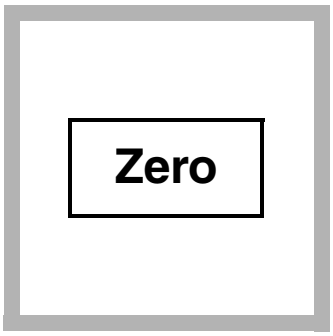
Select program **76 Chlor Diox DPD**.

Touch **Start**.

2. Fill a round sample cell with 10 mL of sample and cap. (This is the blank.)

Fill a second round cell with 10 mL of sample and cap. (This is the prepared sample).

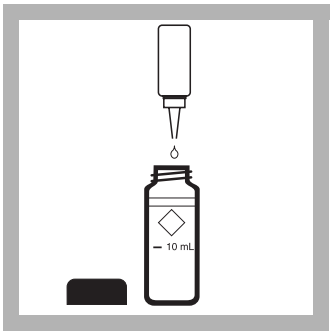
3. Wipe the blank and place it into the cell holder.



4. Touch **Zero**.

The display will show:

0.00 mg/L ClO_2



5. Add four drops of Glycine Reagent to the sample. Swirl to mix.

6. Add the contents of one DPD Free Chlorine Powder Pillow to the prepared sample cell.

Swirl the sample for 20 seconds to mix.

7. Wait 30 seconds for any undissolved powder to settle.

Immediately proceed to *step 8*.

8. Within one minute of adding the reagent, wipe the sample cell and place it into the cell holder.

Touch **Read**.

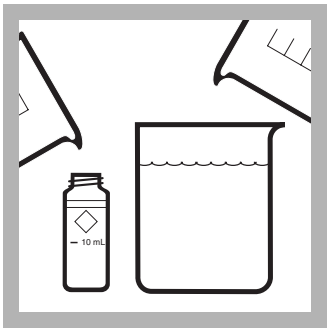
Results will appear in mg/L ClO_2.

AccuVac® Ampul — Method 10126

1. Touch **Hach Programs**.

Select program **77 Chlor Diox, DPD AV**.

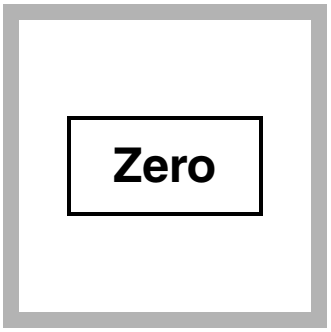
Touch **Start**.



2. Fill a round sample cell with 10-mL of sample. (This is the blank.)

Fill a 50-mL beaker with 40 mL of sample.

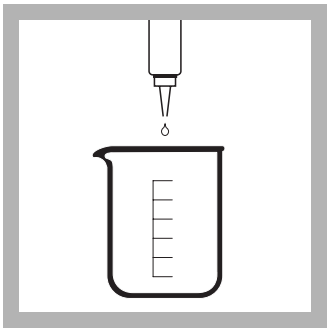
3. Wipe the blank and place it into the cell holder.



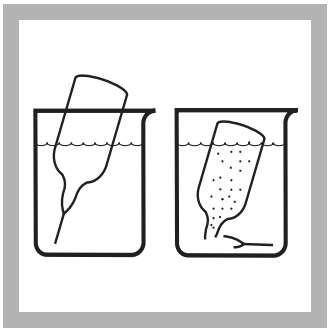
4. Touch **Zero**.

The display will show:

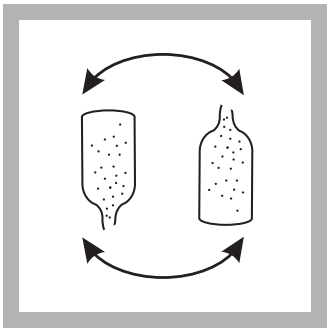
0.00 mg/L ClO_2



5. Add 16 drops of Glycine Reagent to the sample in the beaker. Swirl gently to mix.



6. Fill a DPD Free Chlorine Reagent AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



7. Quickly invert the ampule several times to mix. Wait 30 seconds for any undissolved powder to settle.

8. Within one minute of adding the sample, wipe the ampule and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L ClO_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide (Cat. No. 1045-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions).*
Alkalinity	Greater than 250 mg/L $CaCO_3$. Color may not develop fully or may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for the volume addition (see *Section 3.1.3 Correcting for Volume Additions*).
Bromine, Br_2	Interferes at all levels.
Chlorine, Cl_2	May interfere at levels greater than 6 mg/L. Additional glycine may be able to compensate for this interference.
Chloramines, organic	May interfere.
Flocculating agents	High levels of most flocculating agents can be tolerated. This tolerance is decreased if chlorine is present. See the information about metals in this table. In the presence of 0.6 mg/L Cl_2, $Al(SO_4)_3$ (< 500 mg/L) and $FeCl_2$ (<200 mg/L) may be tolerated.
Hardness	No effect at less than 1,000 mg/L as $CaCO_3$.
Iodine, I_2	Interferes at all levels.
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	Oxidized manganese interferes at all levels. Oxidized chromium interferes at levels greater than 2 mg/L. To remove the interferences: **1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide (30 g/L) (Cat. No. 343-32) to a 25-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops Sodium Arsenite* (5 g/L) (Cat. No. 1047-32) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. Subtract the result of this test from the original analysis to obtain the correct chlorine dioxide concentration.

Interfering Substance	Interference Levels and Treatments
Metals	Various metals may interfere by combining with the glycine needed to remove the chlorine interference. Metal interference is limited except when chlorine is present. In the presence of 0.6 mg/L Cl_2, both copper (>10 mg/L) and nickel (>50 mg/L) interfere. Other metals may also interfere, depending on their ability to prevent glycine from reacting with any Cl_2 in the sample. It may be necessary to add more glycine to overcome this interference.
Monochloramine	Causes a gradual drift to higher readings. When read within 1 minute after reagent addition, 3 mg/L monochloramine causes less than a 0.1 mg/L ClO_2 increase in the reading.
Ozone	Interferes at levels greater than 1.5 mg/L.
Peroxides	May interfere.
Extreme sample pH	Adjust to pH 6–7. See *Section 3.3 Interferences.*
Highly buffered samples	Adjust to pH 6–7. See *Section 3.3 Interferences.*

* Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See *Section 4* for further information on proper disposal of these materials.

Sample Collection, Storage, and Preservation

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine dioxide analysis immediately.

Accuracy Check

Because chlorine dioxide is difficult and hazardous to produce, check the DPD and glycine reagents by using chlorine standards. Proceed as follows:

1. Prepare a 1-mg/L free chlorine standard using Method 1 or 2, below:

Method 1

a. Use Free Chlorine Standard (Cat. No. 14268-10).

b. Determine the concentration of the standard from the certificate of analysis shipped with the standard (50–75 mg/L). Calculate the volume of standard needed as follows:

mL standard needed = 100 ÷ standard concentration

c. Pipet the volume of standard needed into a 100-mL volumetric flask. Dilute to the line with chlorine-demand-free deionized water. Invert to mix.

Method 2

a. Dilute 1 drop of 5% chlorine bleach in 1 liter of chlorine-demand-free deionized water. Use this as the standard.

b. Verify the standard's concentration using the Hach Free Chlorine Method 8021.

c. Perform the chlorine dioxide test on the standard without adding glycine.

d. For program 76, the chlorine dioxide reading should be 2.35 times greater than the chlorine result. For program 77, the chlorine dioxide reading should be 2.34 times greater than the chlorine result. If so, this verifies the DPD and the instrument are functioning properly.

e. Repeat the chlorine dioxide test on the chlorine standard, including the glycine addition. The reading should be less than 0.10 mg/L. This verifies that the glycine is eliminating free chlorine interference.

Method Performance

Precision

Standard: 1.00 mg/L ClO_2

Program	95% Confidence Limits of Distribution
76	0.95–1.05 mg/L ClO_2
77	0.95–1.05 mg/L ClO_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
76	Entire range	0.010	0.04 mg/L ClO_2
77	Entire range	0.010	0.04 mg/L ClO_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chlorine dioxide reacts with DPD (N, N-diethyl-p-phenylenediamine) to the extent of one-fifth of its total available chlorine content corresponding to reduction of chlorine dioxide to chlorite to form a pink color. The color intensity is proportional to the ClO_2 in the sample. Chlorine interference is eliminated by adding glycine, which converts free chlorine to chloroaminoacetic acid, but has no effect on chlorine dioxide at the test pH. Test results are measured at 530 nm.

Chlorine Dioxide

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Chlorine Dioxide DPD/Glycine Reagent Set (100 tests)			27709-00
Includes:			
(1) DPD Free Chlorine Reagent Powder Pillows, 10-mL	1 pillow	100/pkg	21055-69
(1) Glycine Reagent	4 drops	29 mL	27621-33
or			
Chlorine Dioxide DPD/Glycine AccuVac® Ampul Reagent Set (25 tests)			27710-00
Includes:			
(1) DPD Free Chlorine Reagent AccuVac® Ampuls	1 ampule	25/pkg	25020-25
(1) Glycine Reagent	16 drops	29 mL	27621-33

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Beaker, 50-mL	1	each	500-41

Required Standards

Chlorine Standard Solution, 10-mL Voluette® Ampule, 50–75 mg/L		16/pkg	14268-10
Water, deionized		4 liters	272-56

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Chlorine Dioxide

Amaranth Method*
(20 to 500 µg/L)

Scope and Application: For water, drinking water

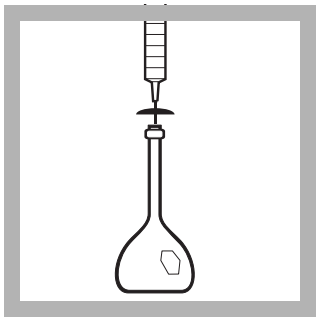
* This method is under license of Elf Atofina. Reagent sets for this method are only available in Europe.

Tips and Techniques

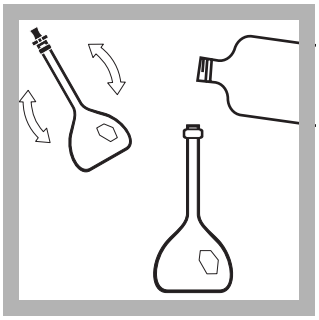
- Analyze samples immediately because chlorine dioxide is unstable and volatile. See *Sample Collection, Storage and Preservation*.
- For most accurate results, analyze each portion of sample at the same temperature.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- A TenSette® pipet (Cat. No. 19700-01) may be used to dispense Chlorine Dioxide Reagent A.

Amaranth Method

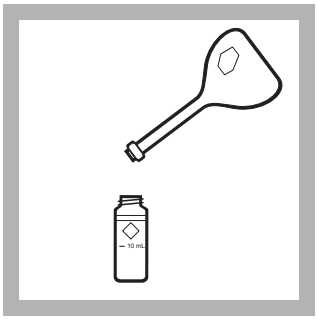
1. Touch **Hach Programs**. Select program **78 ClO_2 Amaranth**. Touch **Start**.



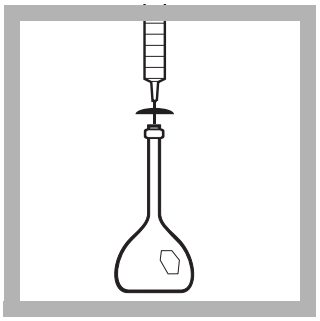
2. Using the syringe and needle provided, add 1.0 mL of Chlorine Dioxide Reagent A into a 25-mL volumetric flask.



3. Fill the volumetric flask to the mark with deionized water. Stopper. Invert several times to mix.

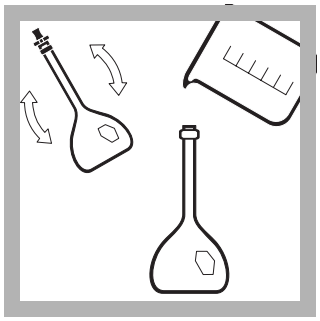


4. Pour 10 mL from the volumetric flask into a 10 mL sample cell. (This is the blank.)

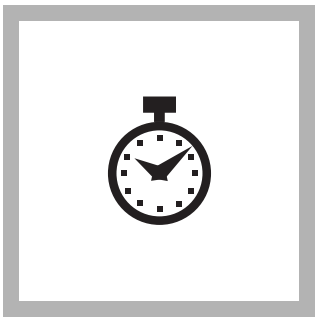


5. Add 1.0 mL of Chlorine Dioxide Reagent A into a second 25-mL volumetric flask.

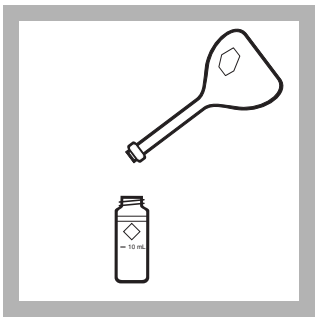
Note: *Use a volumetric pipet and pipet filler or a TenSette Pipet to add this reagent.*



6. Fill the second volumetric flask to the mark with the sample. Stopper. Invert several times to mix.

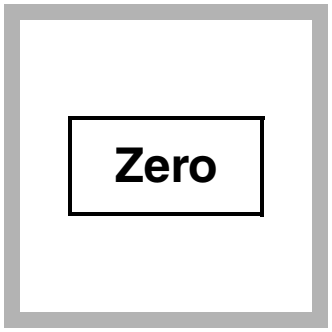


7. Touch the timer icon. Touch **OK**. A 1-minute reaction period will begin.



8. Pour 10 mL from the second volumetric flask into a second sample cell. (This is the sample.)

9. Wipe the blank and place it into the cell holder.

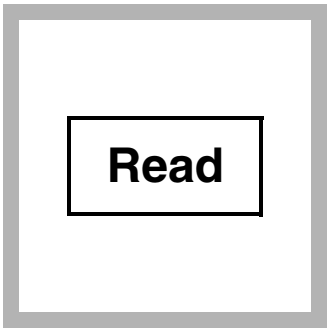


10. Touch **Zero**.

The display will show:

0 µg/L ClO_2

11. When the timer beeps, wipe the prepared sample and place it into the cell holder.



12. Touch Read.

Results will appear in µg/L ClO_2.

Note: *The display will show* **Underrange** *or* **Overrange**, *respectively, if the value is outside the range of the method.*

Interferences

Interfering Substance	Interference Levels and Treatments
ClO^-	Greater than 2.0mg/L
ClO_2^-	Greater than 2.0 mg/L
ClO_3^-	Greater than 2.0 mg/L
CrO_4^{2-}	Greater than 0.2 mg/L
Fe^{3+}	Greater than 0.5 mg/L
Hardness	Greater than 1000 mg/L
Ozone	Greater than 0.5 mg/L
Turbidity	Greater than 1000 NTU

Sample Collection, Storage and Preservation

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine dioxide analysis immediately.

Accuracy Check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 20th ed., under the headings "Stock chlorine dioxide solution" and "Standard chlorine dioxide solution" (pp 4–74 and 4–75). Prepare a 0.25-mg/L (250-µg/L) chlorine dioxide standard.

Method Performance

Precision

Standard: 250 µg/L ClO_2

Program	95% Confidence Limits of Distribution
78	192–308 µg/L ClO_2

See *Section 3.4.3 Precision* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	24 µg/L ClO_2

See *Section 3.4.5 Sensitivity* for more information.

Summary of Method

Chlorine dioxide (ClO_2) is determined by its combination with Amaranth. Color intensity decreases as the level of Chlorine dioxide increase. Test results are measured at 521 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No
Chlorine Dioxide Reagent A Set* (100 Tests)	1	100/pkg	LYW 240
Chlorine Dioxide Tool Set			LZC 140
Includes:			
(2) Volumetric Flask, Plastic, 25-mL	2	each	N/A
(1) Syringe, 1-mL (with needle)	1	each	N/A

Required Apparatus

Cylinder, graduated mixing, 50-mL	2	each	1896-41
Pipet, volumetric, Class A, 1.00-mL	1	each	14515-35
Pipet Filler, safety bulb	1	each	14651-00
Sample cells, 10-mL, w/cap	2	6/pkg	24276-06

*Available only in Europe.

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Chlorine Dioxide

Method 8065

Chlorophenol Red Method*

LR (0.01 to 1.00 mg/L)

Scope and Application: For water and wastewater

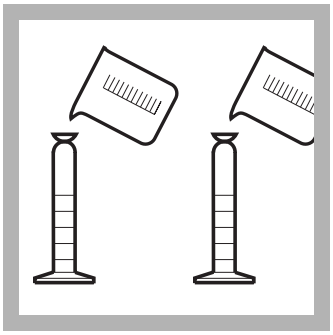
* Adapted from Harp, Klein, and Schoonover, *Jour. Amer. Water Works Assn.*, 73 387–388 (1981).

Tips and Techniques

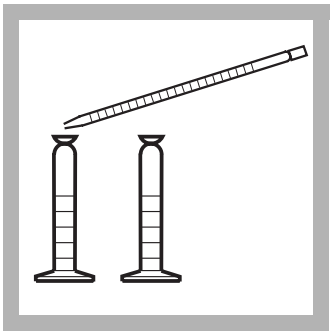
- Analyze samples immediately because chlorine dioxide is unstable and volatile. See *Sample Collection, Storage and Preservation*.
- For most accurate results, analyze each portion of sample at the same temperature.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- A TenSette® pipet (Cat. No. 19700-01) may be used to dispense Chlorine Dioxide Reagent 1 and Chlorine Dioxide Reagent 3.

Powder Pillows — Method 8065

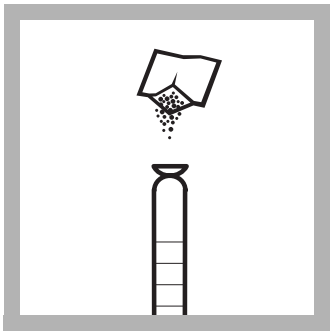
1. Touch **Hach Programs**.
Select program **72 Chlor Diox, CPR LR**.
Touch **Start**.



2. Fill two 50-mL mixing cylinders to the 50-mL mark with sample.

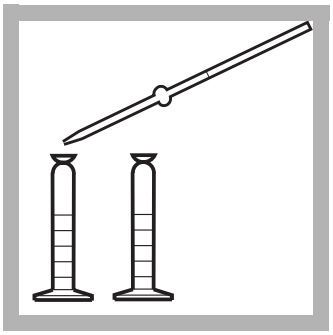


3. Use a volumetric pipet and pipet filler to add 1.0 mL of Chlorine Dioxide Reagent 1 to each cylinder. Stopper. Invert several times to mix.

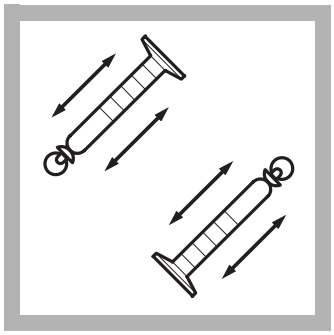


4. Add the contents of one Dechlorinating Reagent Powder Pillow to one cylinder. (This is the blank).
Stopper and invert several times until dissolved.

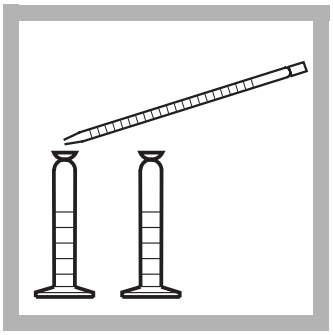
Note: *The second cylinder, which does* ***not*** *receive dechlorinating reagent, is the prepared sample.*



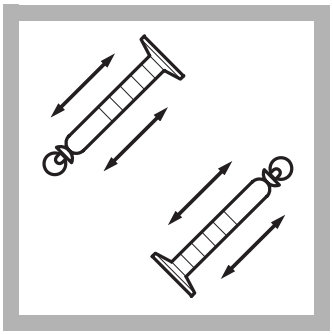
5. Use a Class A pipet to add exactly 1.00 mL of Chlorine Dioxide Reagent 2 to each cylinder. Stopper.



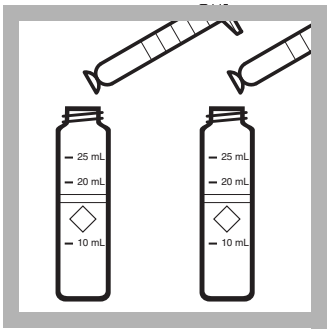
6. Invert several times to mix.



7. Use a volumetric pipet and pipet filler to add 1.0 mL of Chlorine Dioxide Reagent 3 to each cylinder. Stopper.

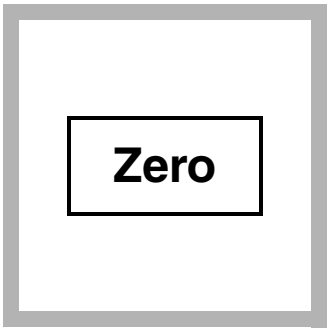


8. Invert several times to mix.



9. Pour 25 mL from each cylinder into round sample cells.

10. Wipe the blank and place it into the cell holder.



11. Touch **Zero**.

The display will show:

0.00 mg/L ClO_2

12. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L ClO_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Highly acidic or alkaline water	May require 2.0 mL each of Chlorine Dioxide Reagent 1 and Chlorine Dioxide Reagent 3 instead of 1.0 mL
ClO^-	Greater than 5.5 mg/L
ClO_2^-	Greater than 6 mg/L
ClO_3^-	Greater than 6 mg/L
CrO_4^{2-}	Greater than 3.6 mg/L
Fe^{3+}	Greater than 5 mg/L
Hardness	Greater than 1000 mg/L
Ozone	Greater than 0.5 mg/L
Turbidity	Greater than 1000 NTU

Sample Collection, Storage and Preservation

Analyze samples for chlorine dioxide immediately after collection. Chlorine dioxide is a strong oxidizing agent and is unstable in natural waters. It reacts rapidly with various inorganic compounds, but oxidizes organic compounds more slowly. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine dioxide in water.

Avoid plastic containers since these may have a large chlorine dioxide demand. Pretreat glass sample containers to remove any chlorine or chlorine dioxide demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pretreatment is necessary.

A common error in testing for chlorine dioxide is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine dioxide analysis immediately.

Accuracy Check

Standard Solution Method

Preparing chlorine dioxide standards is difficult and dangerous. In addition, these standards are both explosive and volatile! Only a trained chemist should prepare the standards using appropriate safety equipment and precautions. Hach does not recommend preparation of chlorine dioxide standards. If independent standard preparation is required, please see the instructions in *Standard Methods for the Examination of Water and Wastewater*, 20th ed., under the headings “Stock chlorine dioxide solution” and “Standard chlorine dioxide solution” (pp 4–74 and 4–75). Prepare a 0.50-mg/L chlorine dioxide standard.

Method Performance

Precision

Standard: 0.50 mg/L ClO_2

Program	95% Confidence Limits of Distribution
72	0.49–0.51 mg/L ClO_2

See *Section 3.4.3 Precision* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
0.00 mg/L	0.010	0.01 mg/L ClO_2
0.50 mg/L	0.010	0.01 mg/L ClO_2
1.00 mg/L	0.010	0.02 mg/L ClO_2

See *Section 3.4.5 Sensitivity* for more information.

Summary of Method

Chlorine Dioxide (ClO_2) is determined by its combination with chlorophenol red at pH 5.2 to form a colorless complex. The net effect is bleaching of the color in an amount proportional to the chlorine dioxide concentration. The method is specific for ClO_2 and is unreactive to other active chlorine or moderate oxidizing compounds. Test results are measured at 575 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No
Chlorine Dioxide Reagent Set (100 Tests)			22423-00
Includes:			
(2) Chlorine Dioxide Reagent 1	2 mL	100 mL	20700-42
(2) Chlorine Dioxide Reagent 2	2 mL	100 mL	20701-42
(2) Chlorine Dioxide Reagent 3	2 mL	100 mL	20702-42
(1) Dechlorinating Reagent Powder Pillows	1 pillow	100/pkg	14363-69

Required Apparatus

Cylinder, graduated mixing, 50-mL	2	each	1896-41
Pipet, volumetric, Class A, 1.00-mL	1	each	14515-35
Pipet Filler, safety bulb	1	each	14651-00
Sample cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

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★Method 8021 — DPD Method*

Powder Pillows or AccuVac® Ampuls — (0.02 to 2.00 mg/L)

Scope and Application: For testing free chlorine (hypochlorous acid and hypochlorite ion) in water, treated waters, estuary, and seawater. USEPA accepted for reporting for drinking water analyses.**

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Procedure is equivalent to USEPA method 330.5 and Standard Method 4500-Cl G for drinking water.

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- After adding reagent to the sample cell, a pink color will develop if free chlorine is present.
- If the test overranges, dilute the sample with a known volume of high quality, chlorine demand-free water (Cat. No. 26415-49) and repeat the test. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. See *Section 2.7 Sample Dilution* on page *21*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows — Method 8021

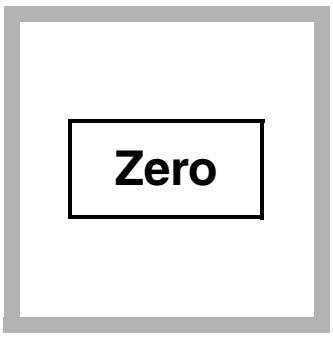
1. Touch **Hach Programs**.

Select program **80 Chlor. F & T**.

Touch **Start**.

2. Fill a round sample cell with 10 mL of sample. (This is the blank.)

3. Wipe the blank and place it into the cell holder.



4. Touch **Zero**.

The display will show:

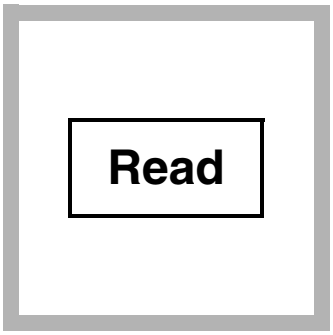
0.00 mg/L Cl_2

5. Fill a second round cell with 10 mL of sample.

6. Add the contents of one DPD Free Chlorine Powder Pillow to the sample cell. (This is the prepared sample).

Swirl the sample cell for 20 seconds to mix. Proceed to step 7 immediately.

7. Within one minute of adding the reagent, place the prepared sample into the cell holder.



8. Touch **Read**.

Results will appear in mg/L Cl_2.

AccuVac® Ampul — Method 8021

1. Touch

Hach Programs.

Select program

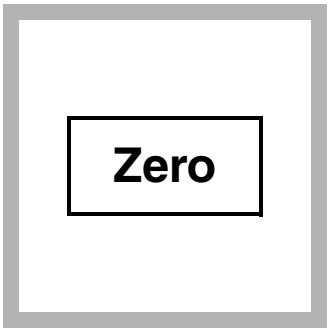
85 Chlor. F & T AV.

Touch **Start**.

2. Fill a round sample cell with 10-mL of sample. (This is the blank.)

Note: *Collect at least 40 mL of sample in a 50-mL beaker.*

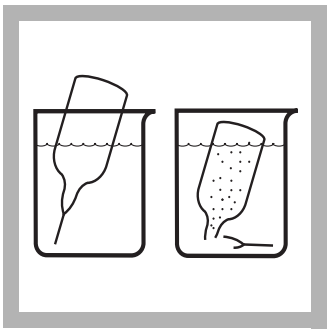
3. Wipe the blank and place it into the cell holder.



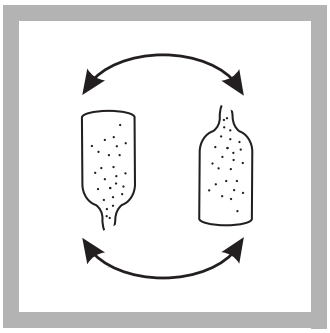
4. Touch **Zero**.

The display will show:

0.00 mg/L Cl_2

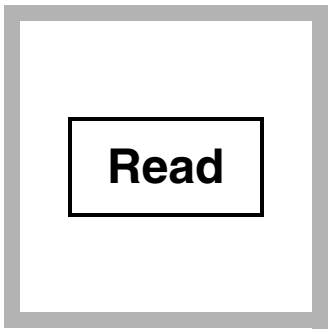


5. Fill a DPD Free Chlorine Reagent AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



6. Quickly invert the ampule several times to mix. Wipe off any liquid or fingerprints.

7. Within one minute of sample addition, wipe the AccuVac Ampul and place it into the cell holder.



8. Touch **Read**.

Results will appear in mg/L Cl_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide (Cat. No. 1045-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as $CaCO_3$
Iodine, I_2	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide (30-g/L) (Cat. No. 343-32) to a 10-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops Sodium Arsenite* (5-g/L) (Cat. No. 1047-32) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Monochloramine	Causes a gradual drift to higher readings. When read within 1 minute after reagent addition, 3 mg/L monochloramine causes less than a 0.1 mg/L increase in the reading.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or Highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid, 1.000 N, Cat. No. 1270-32) or base (Sodium Hydroxide, 1.00 N, Cat. No. 1045-32).

* Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See *Section 4* for further information on proper disposal of these materials.

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, the carefully fill to the 10-mL mark. Perform the chlorine analysis immediately.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the Chlorine Voluette® Ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After the values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L Cl_2.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 10 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.

Note: *For AccuVac® Ampuls, fill three mixing cylinders (Cat. No. 1896-41) with 50 mL of sample and spike with 0.4 mL, 0.8 mL, and 1.2 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision
Standard: 1.07 mg/L Cl_2

Program	95% Confidence Limits of Distribution
80	1.00–1.14 mg/L Cl_2
85	1.03–1.11 mg/L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
80	Entire range	0.010	0.02 mg/L Cl_2
85	Entire range	0.010	0.02 mg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator to form a pink color, the intensity of which is proportional to the chlorine concentration. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
DPD Free Chlorine Reagent Powder Pillows, 10-mL	1 pillow	100/pkg	21055-69
or			
DPD Free Chlorine Reagent AccuVac® Ampuls	1 ampul	25/pkg	25020-25

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cell, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Chlorine Standard Solution, 2-mL Voluette® Ampule, 20–30 mg/L		20/pkg	26300-20

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Chlorine, Free

Method 10069 **DPD Method***

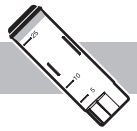
Multi-pathlength Cell **HR (0.1 to 10.0 mg/L as Cl_2)**

Scope and Application: For testing higher levels of free chlorine (hypochlorous acid and hypochlorite ion) in drinking water, cooling water, and industrial process waters

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- After adding the DPD reagent, a pink color will develop if chlorine is present.
- If the chlorine concentration is less than 2 mg/L, use Method 8021, program number 80.
- Install the Multi-pathlength Cell into the adapter oriented for the short (1-cm) pathlength (the long pathlength is perpendicular to the front of the instrument).



DR/2400 Multi-Path Cell Method 10069

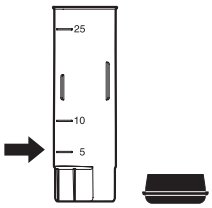
1. Touch

Hach Programs.

Select program

88 Chlor. F&T HR.

Touch **Start**.



2. Fill the multi-pathlength cell to the 5-mL line with sample.

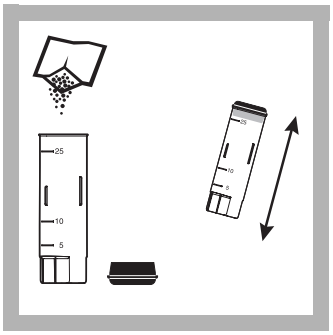
3. Install the adapter. Wipe the cell. Place the cell into the cell adapter and orient it for the short pathlength.

Note: *See Section 2.6.1 Installing Sample Cell Adapters in the Instrument Manual for adapter installation details.*

4. Touch **Zero**.

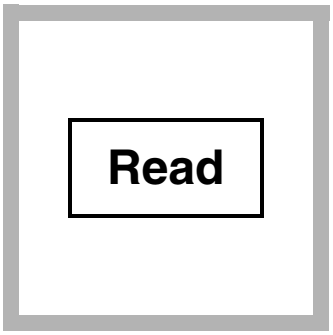
The display will show:

0.0 mg/L Cl_2



5. Remove the cell from the adapter and add the contents of one DPD Free Chlorine powder pillow to the sample. Cap and shake the cell about 20 seconds to dissolve.

6. Place the prepared sample into the adapter.



7. Touch **Read**.

Results will appear in mg/L Cl_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with1 N Sodium Hydroxide (Cat. No. 1045-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions*).
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions*).
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Iodine, I_2	Interferes at all levels
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	1. Adjust sample pH to 6–7 with 1.000 N Sulfuric Acid (Cat. No. 1270-32). 2. Add 2 drops Potassium Iodide (30 g/L) (Cat. No. 343-32) to a 5-mL sample. 3. Mix and wait 1 minute. 4. Add 2 drops Sodium Arsenite* (5 g/L) (Cat. No. 1047-32) and mix. 5. Analyze the treated sample as described in the procedure above. 6. Subtract the result of this test from the original analysis to obtain the correct concentration.
Monochloramine (NH_2Cl)	For conventional free chlorine disinfection (beyond the "breakpoint"), typical monochloramine concentrations are very low. If monochloramine is present in the sample, its interference in the free chlorine test is dependent on sample temperature, relative concentration of monochloramine to free chlorine, and the time required to perform the analysis. Typical interference levels of NH_2Cl (1 minute test time, interference as mg/L Cl_2):

NH_2Cl Level	Sample Temperature °C (°F)			
	5 (41)	10 (50)	20 (68)	30 (86)
1.2 mg/L	+0.15	0.19	0.30	0.29
2.5 mg/L	+0.35	0.38	0.55	0.61
3.5 mg/L	+0.38	0.56	0.69	0.73
5.0 mg/L	+0.68	0.75	0.93	1.05

Interfering Substance	Interference Levels and Treatments
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid, Cat. No. 1270-32) or base (Sodium Hydroxide, Cat. No. 1045-32).

* Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). For information on pollution prevention and waste management, refer to *Section 4*.

Sampling and Storage

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and reacts rapidly with various compounds. Many factors such as sunlight, pH, temperature and sample composition will influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over to the free chlorine test, monochloramine could interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 5-mL mark. Proceed with the chlorine test immediately.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the chlorine voluette ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a High Range Chlorine PourRite® Ampule Standard, 50–70 mg/L.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 5-mL of sample. Using the TenSette® Pipet, add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to each sample and mix thoroughly.

6. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

See *Section 3.2.2 Standard Additions* for more information.

Method Performance

Precision

Standard: 5.6 mg/L Cl_2

Program	95% Confidence Limits of Distribution
88	5.5–5.7 mg/L

See *Section 3.4.3 Precision* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.1 mg/L Cl_2

See *Section 3.4.5 Sensitivity* for more information.

Summary of Method

The range of analysis using the DPD method for free chlorine can be extended by adding more indicator in proportion to sample volume. Thus, a larger fill powder pillow of DPD Free Chlorine Reagent is added to a 5-mL sample portion.

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N, N-diethyl-p-phenylenediamine) indicator to form a magenta color which is proportional to the chlorine concentration.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
DPD Free Chlorine Reagent Powder Pillows	1 pillow	100/pkg	14070-99

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cell, sample, multi-pathlength	2	6/pkg	59405-06

Required Standards

Chlorine Standard Solution, 2-mL PourRite® Ampules, 50–75 mg/L		20/pkg	14268-20

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FAX: (970) 669-2932

Chlorine, Free

Method 10059

DPD Rapid Liquid Method*

Pour-Thru™ Cell

(0.02 to 2.00 mg/L)

Scope and Application: For treated water.

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- Clean the Pour-Thru cell and all labware as specified in *Treating Analysis Labware on page 4*.
- See *Reagent Preparation on page 3* for instructions on preparing the indicator solution.
- Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

DR/2400 Pour-Thru Cell Method 10059

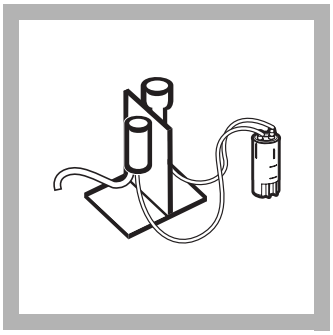
1. Touch

Hach Programs.

Select program

82 Chlor. F & T RL.

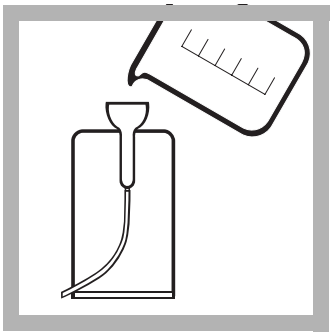
Touch **Start**.



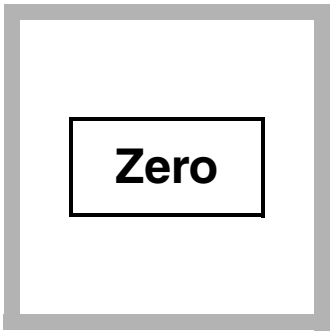
2. Install the Pour-Thru Cell and multi-pathlength cell adapter in the sample cell compartment. Use the 25-mm cell pathlength.

Note: *See Section 2.6.1 in the Instrument manual for installation details.*

Flush with 50-mL of deionized water.



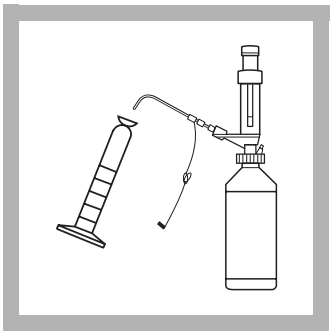
3. Pour approximately 50 mL of sample into the Pour-Thru Cell.



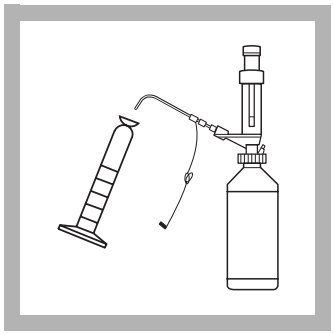
4. When the flow stops, touch **Zero**.

The display will show:

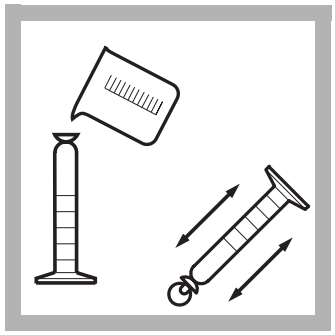
0.00 mg/L Cl_2



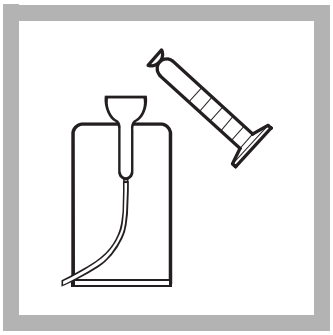
5. Add 1.0 mL of Free Chlorine Buffer Solution to a clean, dry 100-mL glass mixing cylinder using the Repipet Jr. Dispenser.



6. Add 1.0 mL of prepared Free Chlorine Indicator Solution to the same mixing cylinder using the Repipet Jr. Dispenser. Swirl to mix the reagents. Proceed to step 7 immediately.



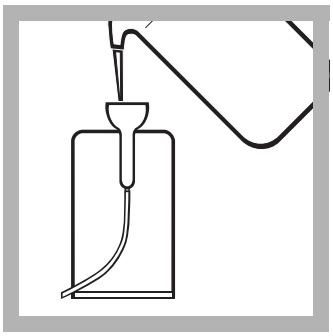
7. Carefully fill the mixing cylinder to the 80-mL mark with sample. Stopper the cylinder and gently invert it twice to mix. Proceed to step 8 immediately.



8. Fill the funnel of the Pour-Thru Cell with the reacted sample from the mixing cylinder.

Note: *It is not necessary to pour the entire sample into the Pour-Thru Cell; approximately half of the sample may be discarded.*

After the flow stops, touch **Read**. Results will appear in mg/L Cl_2.



9. Flush the Pour-Thru Cell with at least 50-mL of deionized water immediately after use

Reagent Preparation

The Free Chlorine Indicator Solution must be prepared before use. Using a powder funnel, add the contents of one 24 g bottle of DPD Powder (Cat. No. 22972-55) to one 473-mL bottle of Free Chlorine Indicator Solution (Cat. No. 23140-11). Invert several times and swirl until the powder is completely dissolved. A pink color may develop, but should not affect results.

This solution will give accurate results for at least one month after mixing when stored at 20–25 °C (68–77 °C). Write the date of preparation on the Indicator Solution Bottle. Discard any remaining solution after one month. Use of this reagent after one month may result in high reagent blanks and low values at high concentration. Do not combine fresh reagent with previously mixed reagent.

Interferences

Interfering Substance	Interference Levels and Treatments
Alkalinity	Greater than 400 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Bromine, Br_2	Interferes at all levels.
Hardness	Levels below 1000 mg/L as $CaCO_3$ will not interfere.
Iodine, I_2	Interferes at all levels.
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7 with 1.000 N Sulfuric Acid (Cat. No. 1270-32). **2.** Add 9 drops Potassium Iodide (30 g/L) (Cat. No. 343-32) to a 80-mL sample. **3.** Mix and wait 1 minute. **4.** Add 9 drops Sodium Arsenite* (5 g/L) (Cat. No. 1047-32) and mix. **5.** Analyze the treated sample as described in the procedure above. **6.** Subtract the result of this test from the original analysis to obtain the correct concentration.
Monochloramine (NH_2Cl)	Samples containing monochloramine will cause a gradual drift to higher chlorine readings. When read within one minute of reagent addition, 3.0 mg/L monochloramine will cause an increase of less than 0.1 mg/L in the free chlorine reading.
Ozone	Interferes at all levels.

* Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). For information on pollution prevention and waste management, refer to *Section 4*. Refer to the instrument manual for information on modifying the Pour-thru Cell for collecting sample after analysis.

Sampling and Storage

Samples must be analyzed immediately and cannot be preserved for later analysis. A common testing error is introduced if the analyst does not obtain a representative sample. If sampling from a tap, let the water flow for at least five minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Avoid plastic containers since these may have a chlorine demand. Pre-treat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized water.

If sample containers are rinsed thoroughly with deionized water after use, only occasional pretreatment is necessary. A pre-treated BOD bottle with a ground-glass stopper makes an ideal sample container for chlorine analysis.

Treating Analysis Labware

Glassware used in this test must be chlorine demand-free. Fill the 100-mL mixing cylinder and sample container with a dilute solution of chlorine bleach prepared by adding 1 mL of commercial bleach to 1 liter of water. Soak in this solution at least one hour. After soaking, rinse thoroughly with deionized water and allow to dry before use. If the mixing cylinder is thoroughly rinsed with deionized water and allowed to dry after each use, only occasional pretreatment is necessary. Do not use the same mixing cylinder for Free and Total Chlorine analysis.

Treat the Pour-Thru Cell similarly with dilute bleach and let stand for several minutes. Rinse several times with deionized water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N Sulfuric Acid (Cat. No. 2449-53) followed by several rinsings with deionized water.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the chlorine voluette ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the top off a Chlorine Voluette® Ampule Standard Solution, 50 to 75-mg/L Cl_2.
5. Prepare three sample spikes. Use the TenSette® Pipet to add 0.3, 0.6 and 0.9 mL of standard to three 80-mL samples, respectively. Swirl gently to mix.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision

Standard: 1.07 mg/L Cl_2

Program	95% Confidence Limits of Distribution
82	1.05–1.09 mg/L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.02 mg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator to form a red color which is proportional to the chlorine concentration. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Reagent Set			25569-00
Includes:			
DPD Indicator Powder		24 g	22972-55
Free Chlorine Indicator Solution	1 mL	473 mL	23140-11
Free Chlorine Buffer Solution	1 mL	473 mL	23141-11

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cylinder, mixing, glass, 100-mL	1	each	1896-42
Dispenser, fixed volume, 1.0 mL Repipet Jr.	2	each	21113-01
Pour-Thru Cell Kit	1	each	59404-00

Required Standards

Chlorine Standard Solution, Voluette® Ampule, 50-75 mg/L, 10-mL	16/pkg	14268-10
or		
Chlorine Standard Solution, Voluette® Ampule, 50-75 mg/L, 2-mL	20/pkg	14268-20
Water, deionized	4 L	272-56

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3/04 1ed

Chlorine, Free

Method 10102 **DPD Method***

Test 'N Tube™ Vials **(0.09 to 5.00 mg/L)**

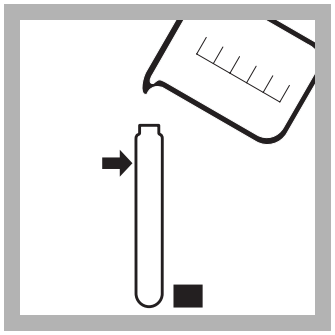
Scope and Application: For testing higher levels of free chlorine (hypochlorous acid and hypochlorite ion) in drinking water, cooling water, and industrial process water.

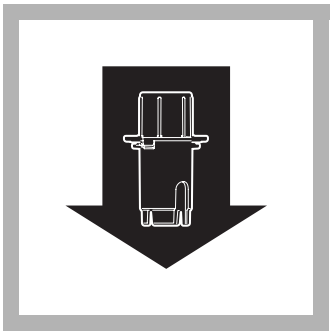
* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

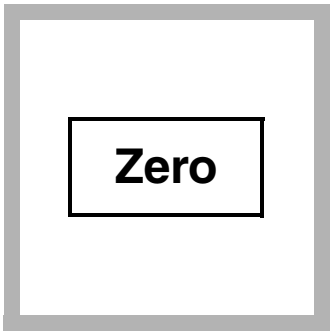
Tips and Techniques

- Analyze samples immediately. Do not preserve samples for later analysis.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- After adding sample to the Test 'N Tube™, a pink color will develop if free chlorine is present.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Test 'N Tube Method 10102







1. Touch

Hach Programs.

Select program

89 Chlor. F&T HR TNT.

Touch **Start**.

2. Fill an empty Test 'N Tube™ vial to the top of the Hach label with sample (This is the blank.)

3. Install the 16-mm cell adapter.

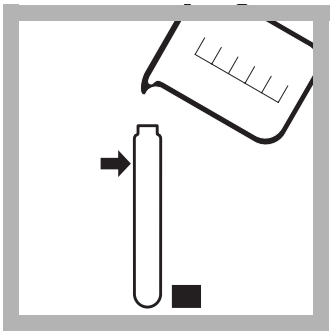
Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Wipe the blank and place it into the adapter.

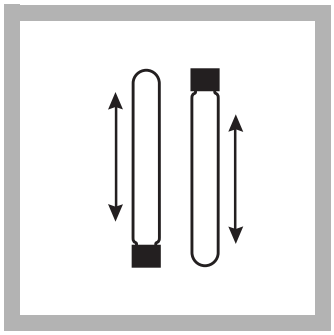
4. Touch **Zero**.

The display will show:

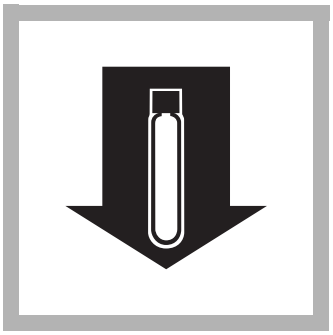
0.00 mg/L Cl_2



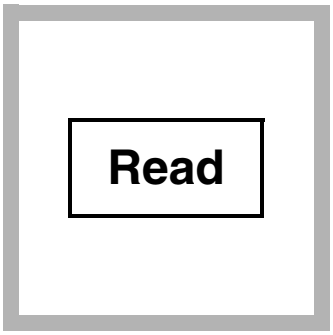
5. Remove the cap from a Free Chlorine DPD Test 'N Tube. Add 10 mL of sample to the tube. (Fill the vial to the top of the Hach label.



6. Cap and invert at least 10 times to dissolve the powder. (This is the prepared sample).



7. Wipe the sample and place it in the adapter.



8. Touch **Read**.

Results will appear in mg/L Cl_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with1 N sodium hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29).*
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as $CaCO_3$
Iodine, I_2	Interferes at all levels
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops potassium iodide (30-g/L) to a 25-mL sample. **3.** Mix and wait 1 minute. **4.** Add 3 drops sodium arsenite* (5-g/L) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration in the sample.

(continued)

<table>
<tr><th>Interfering Substance</th><th>Interference Levels and Treatments</th></tr>
<tr><td>Monochloramine</td><td>For conventional free chlorine disinfection (beyond the breakpoint), typical monochloramine concentrations are very low. If monochloramine is present in the sample, its interference in the free chlorine test depends on the sample temperature, relative amount of monochloramine to free chlorine, and the time required to do the analysis.Typical interference levels of monochloramine in the free chlorine test are listed below (as mg/L Cl_2).
<table>
<tr><th rowspan="2">NH_2Cl (as Cl_2)</th><th colspan="4">Sample Temp. °C (°F)</th></tr>
<tr><th>5 (40)</th><th>10 (50)</th><th>20 (68)</th><th>30 (83)</th></tr>
<tr><td>1.2 mg/L</td><td>+0.15</td><td>0.19</td><td>0.30</td><td>0.29</td></tr>
<tr><td>2.5 mg/L</td><td>+0.35</td><td>0.38</td><td>0.55</td><td>0.61</td></tr>
<tr><td>3.5 mg/L</td><td>+0.38</td><td>0.56</td><td>0.69</td><td>0.73</td></tr>
</table></td></tr>
<tr><td>Ozone, O_3</td><td>Interferes at all levels</td></tr>
<tr><td>Peroxides</td><td>May interfere</td></tr>
<tr><td>Extreme sample pH or Highly buffered samples</td><td>Adjust to pH 6–7 using acid (Sulfuric Acid, 1.000 N, Cat. No. 1270-32) or base (Sodium Hydroxide, 1.00 N, Cat. No. 1045-32).</td></tr>
</table>

* Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by Federal RCRA for arsenic (D004). See *Section 4* of the for further information on proper disposal of these materials.

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

A common error in testing for chlorine is not obtaining an unrepresentative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify that the units displayed are in mg/L.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the standard solution. Touch **OK**. A summary of the Standard Additions procedure will appear. Touch **OK**.

3. Snap the neck off a HR Chlorine PourRite® Ampule Standard, 50–75 mg/L Cl_2.
4. Use the TenSette® Pipet to add 0.1 mL to a 10-mL sample. Mix thoroughly.
5. Analyze the standard addition sample as described in the procedure above. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision

Standard: 2.33 mg/L CL_2

Program	95% Confidence Limits of Distribution
89	2.18–2.48 mg/L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.09 mg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chlorine in the sample as hypochlorous acid or hypochlorite ion (free chlorine or free available chlorine) immediately reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator to form a pink color which is proportional to the chlorine concentration. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Test 'N Tube™ DPD Free Chlorine Reagent	1 vial	50/pkg	21055-45

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Kimwipes	4	280/pkg	20970-00

Required Standards

Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L		20/pkg	14268-20

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Chlorine, Total

★**Method 8167**

DPD Method*

Powder Pillows or AccuVac® Ampuls

(0.02 to 2.00 mg/L)

Scope and Application: For testing residual chlorine and chloramines in water, wastewater, estuary water, and seawater; USEPA-accepted for reporting** for drinking and wastewater analyses.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** Procedure is equivalent to USEPA method 330.5 and Standard Method 4500-Cl G for drinking water and wastewater analyses.

Tips and Techniques

- Samples must be analyzed immediately and cannot be preserved for later analysis.
- After adding the reagent, a pink color will develop if chlorine is present.
- If the test overranges, dilute the sample with a known volume of high quality, chlorine demand-free water (Cat. No. 26415-49), and repeat the test. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. See *Section 2.7 Sample Dilution* on page *21*.
- For chloramination disinfection control, use Method 10172, Chloramine (Mono), Low Range (program number 66) or High Range (program number 67).
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows — Method 8167

1. Touch

Hach Programs.

Select program

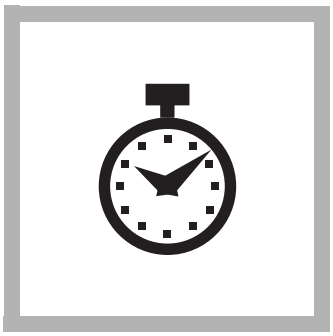
80 Chlor. F&T.

Touch **Start**.

2. Fill a round sample cell with 10 mL of sample.

3. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell. (This is the prepared sample).

Swirl the sample cell for 20 seconds to mix.



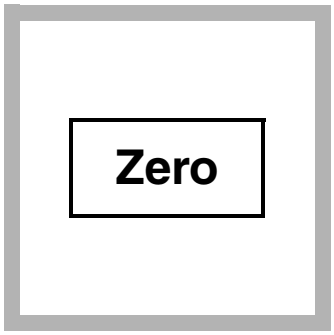
4. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin. Perform s*teps 5* and *6* during this time period.

5. Fill another round sample cell with 10-mL of sample. (This is the blank.)

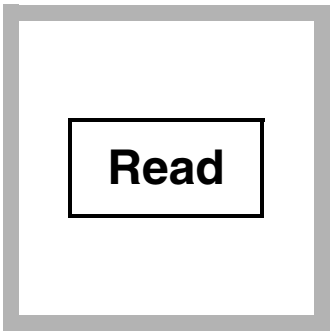
Wipe the sample cell and place it into the cell holder.



6. Touch **Zero**. The display will show:

0.00 mg/L Cl_2

7. Within three minutes after the timer beep, wipe the prepared sample and place it into the cell holder.



8. Touch **Read**.

Results will appear in mg/L Cl_2.

AccuVac® Ampul — Method 8167

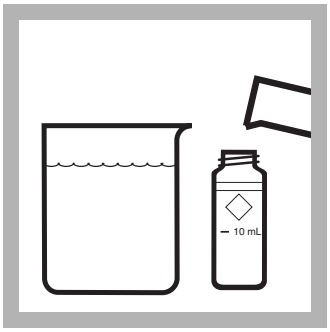
1. Touch

Hach Programs.

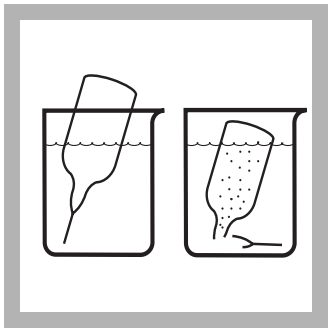
Select program

85 Chlor. F&T AV.

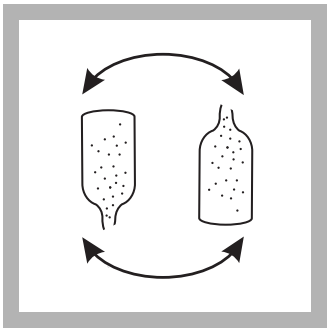
Touch **Start**.



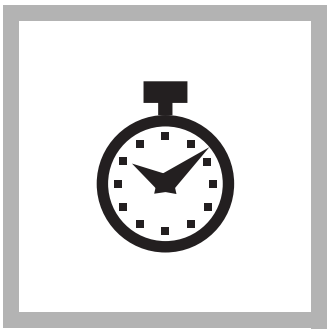
2. Fill a round sample cell with 10-mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.



3. Fill a DPD Total Chlorine Reagent AccuVac® Ampul with sample. Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampule several times to mix. Wipe off any liquid or fingerprints.

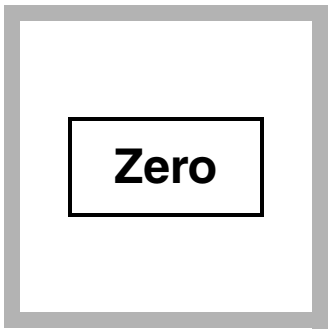


5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin. Perform steps 6 and 7 during this time period.

6. Wipe the blank and place it into the cell holder.



7. Touch **Zero**. The display will show:

0.00 mg/L Cl_2

8. Within three minutes after the timer beep, wipe the AccuVac Ampul and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L Cl_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide (Cat. No. 1045-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (See *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Alkalinity	Greater than 300 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (See *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1,000 mg/L as $CaCO_3$
Iodine, I_2	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops potassium iodide (30 g/L) (Cat. No. 343-32) to a 25-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops sodium arsenite* (5 g/L) (Cat. No. 1047-32) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or Highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid, 1.000 N, Cat. No. 1270-32) or base (Sodium Hydroxide, 1.00 N, Cat. No. 1045-32).

* Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See *Section 4* for more information on proper disposal of these materials.

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, the carefully fill to the 10-mL mark. Perform the chlorine analysis immediately.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the Chlorine Voluette® Ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a LR Chlorine Voluette® Ampule Standard, 25–30 mg/L Cl_2.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 10 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.

Note: *For AccuVac® Ampuls, fill three mixing cylinders (Cat. No. 1896-41) with 50-mL of sample and spike with 0.4 mL, 0.8 mL, and 1.2 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision
Standard: 1.07 mg/L Cl_2

Program	95% Confidence Limits of Distribution
80	1.05–1.09 mg/L Cl_2
85	1.03–1.11 mg/L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
80	Entire range	0.010	0.02 mg/L Cl_2
85	Entire range	0.010	0.02 mg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can exist in the same water and be determined together as the total chlorine. Free chlorine is present as hypochlorous acid or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives. The combined chlorine oxidizes iodide in the reagent to iodine. The iodine and free chlorine reacts with DPD (N,N-diethyl-p-phenylenediamine) to form a red color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
DPD Total Chlorine Reagent AccuVac® Ampuls	1 ampul	25/pkg	25030-25
or DPD Total Chlorine Reagent Powder Pillows, 10-mL	1 pillow	100/pkg	21056-69
Water, deionized		4 liters	272-56

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Chlorine Standard Solution, 2-mL Voluette® Ampule, 20–30 mg/L		20/pkg	26300-20

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On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Chlorine, Total

Method 10070 **DPD Method***

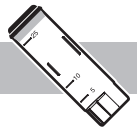
Multi-pathlength Cell **HR (0.1 to 10.0 mg/L as Cl_2)**

Scope and Application: For testing higher levels of total chlorine (free and combined) in drinking water, cooling water, industrial process waters, or treated wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- If chlorine is present, a pink color will develop after adding DPD Total Chlorine Reagent.
- If the chlorine concentration is typically less than 2 mg/L, use Method 8167, program number 80.
- Install the Multi-pathlength Cell into the adapter oriented for the short (1-cm) pathlength (the long pathlength is perpendicular to the front of the instrument).



DR/2400 Multi-Path Cell Method 10070

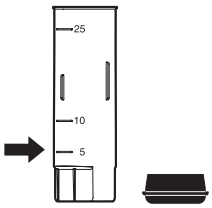
1. Touch

Hach Programs.

Select program

88 Chlor. F&T HR

Touch **Start**.



2. Fill the multi-pathlength cell to the 5-mL line with sample.

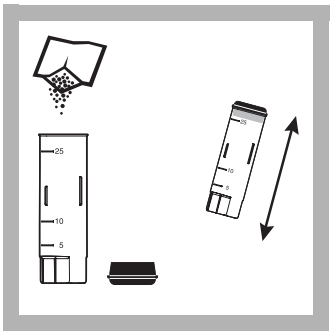
3. Install the adapter. Wipe the cell. Place the cell into the cell adapter and orient it for the short pathlength.

Note: *See Section 2.6.1 Installing Sample Cell Adapters in the Instrument Manual for adapter installation details.*

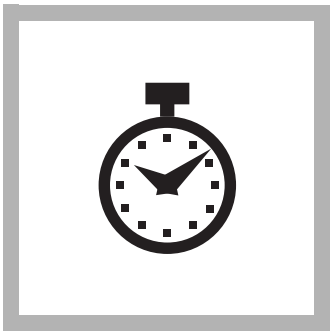
4. Touch **Zero**.

The display will show:

0.0 mg/L Cl_2



5. Remove the cell from the adapter and add the contents of one DPD Total Chlorine powder pillow to the sample. Cap and shake the cell about 20 seconds to dissolve.

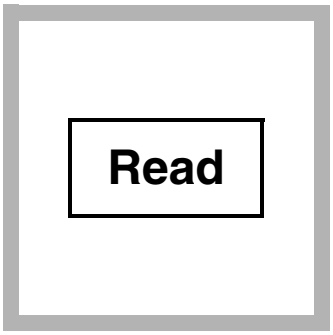


6. Touch the timer icon.

Touch **OK**.

A 3-minute reaction period will begin.

7. After the color has developed fully, wipe the cell and place it into the adapter.



8. Touch **Read**.

Results will appear in mg/L Cl_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with1 N Sodium Hydroxide (Cat. No. 1045-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions*).
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions*).
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Iodine, I_2	Interferes at all levels
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 2 drops Potassium Iodide (30 g/L) (Cat. No. 343-32) to a 5-mL sample. **3.** Mix and wait 1 minute. **4.** Add 2 drops Sodium Arsenite* (5 g/L) (Cat. No. 1047-32) and mix. **5.** Analyze the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or Highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid, 1.000 N, Cat. No. 1270-32) or base (Sodium Hydroxide, 1.00 N, Cat. No. 1045-32).

* Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See *Section 4* for more information on proper disposal of these materials.

Sampling and Storage

Analyze samples for chlorine immediately after collection. Free and combined chlorine are strong oxidizing agents and react rapidly with various compounds. Many factors such as sunlight, pH, temperature and sample composition will influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over to the free chlorine test, monochloramine could interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 5-mL mark. Proceed with the chlorine test immediately.

Method Performance

Precision

Standard: 5.6 mg/L Cl_2

Program	95% Confidence Limits of Distribution
88	5.5–5.7 mg/L Cl_2

See *Section 3.4.3 Precision* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.1 mg/L Cl_2

See *Section 3.4.5 Sensitivity* for more information.

Use Method 8167 to test chlorine concentrations at levels typically less than 2 mg/L or Method 8370 to test chlorine concentrations at levels typically less than 500 µg/L. For more information on determining precision data and method detection limits, refer to *Section 3.4 Method Performance*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the chlorine voluette ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a HR Chlorine PourRite Ampule Standard.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 5-mL of sample. Using the TenSette® Pipet, add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze the standard addition as described in the procedure above. Accept the standard addition by touching **Read**.

See *Section 3.2.2 Standard Additions* for more information.

Summary of Method

The range of analysis using the DPD method for free chlorine can be extended by adding more indicator in proportion to sample volume. Thus, a larger fill powder pillow of DPD Free Chlorine Reagent is added to a 5-mL sample portion.

The combined chlorine oxidizes iodide in the reagent to iodine. The iodine reacts with DPD (N, N-diethyl-p-phenylenediamine) along with free chlorine present in the sample to form a red color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run free chlorine and total chlorine tests. Subtract the results of the free chlorine test from the results of the total chlorine test to obtain combined chlorine.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
DPD Total Chlorine Reagent Powder Pillows	1 pillow	100/pkg	14064-99

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cell, sample, multi-pathlength	2	6/pkg	59405-06

Required Standards

Chlorine Standard Solution, PourRite® Ampules, 50–75 mg/L		20/pkg	14268-20

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Chlorine, Total

Method 10060

DPD Rapid Liquid Method

Pour-Thru Cell

(0.02 to 2.00 mg/L)

Scope and Application: For treated water*

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

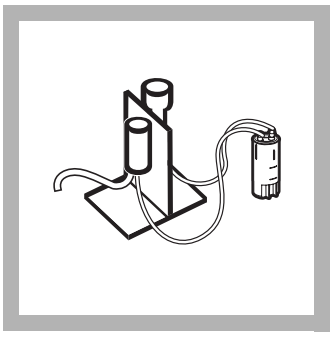
- Analyze samples immediately. Do not preserve for later analysis.
- Clean the Pour-Thru cell and all labware as specified in *Treating Analysis Labware on page 4*.
- See*Reagent Preparation on page 3* for instructions on preparing the indicator solution.
- Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

DR/2400 Pour-Thru Cell Method 10060

1. Touch **Hach Programs**.

Select program **82 Chlor. F&T RL**.

Touch **Start**.

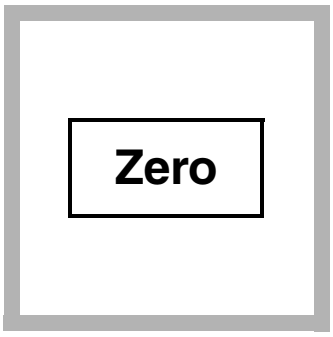


2. Install the Pour-Thru Cell and the multi-pathlength cell adapter in the sample cell compartment. Use the 25-mm cell pathlength.

Note: *See Section 2.6.1 on page 26 in the Instrument manual for installation details.*

Flush with 50-mL of deionized water.

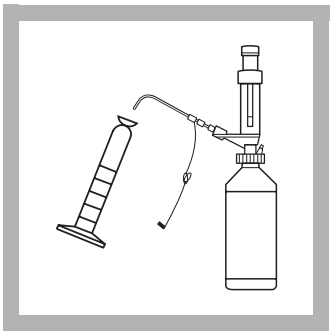
3. Pour approximately 50 mL of sample into the Pour-Thru Cell.



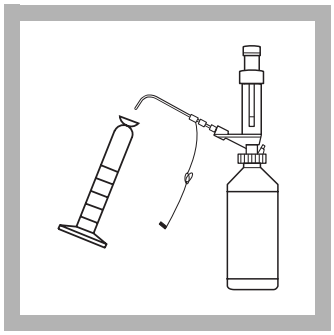
4. When the flow stops, touch **Zero**.

The display will show:

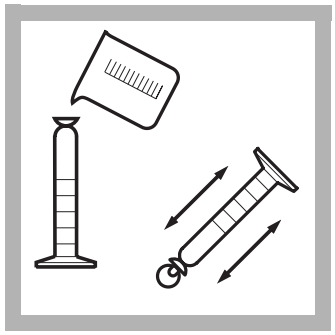
0.00 mg/L Cl_2



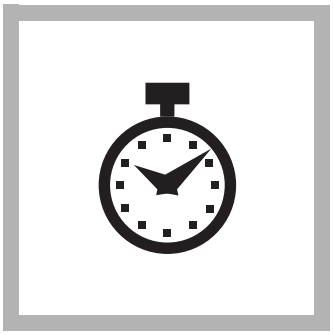
5. Add 1.0 mL of Total Chlorine Buffer Solution to a clean, dry 100-mL glass mixing cylinder using the Repipet Jr. Dispenser.



6. Add 1.0 mL of prepared Total Chlorine Indicator Solution to the same mixing cylinder using the Repipet Jr. Dispenser. Swirl to mix the reagents. Proceed to step 7 immediately.



7. Carefully fill the mixing cylinder to the 80-mL mark with sample. Stopper the cylinder and gently invert it twice to mix. Proceed to step 8 immediately.



8. Touch the timer icon.

Touch **OK**.

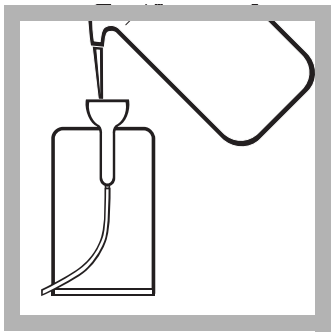
A two-minute reaction period will begin.

Complete steps 9 and 10 within two minutes after the timer beeps.

9. When the timer beeps, fill the funnel of the Pour-Thru Cell with the reacted sample from the mixing cylinder.

Note: *It is not necessary to pour the entire sample into the Pour-Thru Cell; approximately half of the sample may be discarded.*

After the flow stops, touch **Read**. Results will appear in mg/L Cl_2.



10. Flush the Pour-Thru Cell with at least 50-mL of deionized water immediately after use.

Reagent Preparation

The Total Chlorine Indicator Solution must be prepared before use. Using a powder funnel, add the contents of one 24 g bottle of DPD Powder (Cat. No. 22972-55) to one 473-mL bottle of Total Chlorine Indicator Solution (Cat. No. 22634-11). Invert several times and swirl until the powder completely dissolves. A pink color may develop, but this should not affect test results.

This solution will give accurate results for at least one month after mixing when stored at 20–25 °C (68–77 °C). Write the date of preparation on the Indicator Solution Bottle. Discard any remaining solution after one month. Use of this reagent after one month may result in high reagent blanks and low values at high concentration. Do not combine fresh reagent with previously mixed reagent.

Interferences

Interfering Substance	Interference Levels and Treatments
Alkalinity	Greater than 700 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Bromine, Br_2	Interferes at all levels.
Hardness	Levels below 1000 mg/L as $CaCO_3$ will not interfere.
Hexavalent Chromium	Levels greater than 1 mg/L will cause a positive interference.
Iodine, I_2	Interferes at all levels.
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7 with 1.000 N Sulfuric Acid (Cat. No. 1270-32). **2.** Add 9 drops Potassium Iodide (30-g/L) (Cat. No. 343-32) to a 80-mL sample. **3.** Mix and wait 1 minute. **4.** Add 9 drops Sodium Arsenite* (5-g/L) (Cat. No. 1047-32) and mix. **5.** Analyze the treated sample as described in the procedure above. **6.** Subtract the result of this test from the original analysis to obtain the correct concentration.
Ozone	Interferes at all levels.

* Samples treated with sodium arsenite for interferences will be hazardous waste as regulated by the Federal RCRA for arsenic (D004). For information on pollution prevention and waste management, refer to *Section 4*. Refer to the instrument manual for information on modifying the Pour-thru Cell for collecting sample after analysis.

Sampling and Storage

Samples must be analyzed immediately and cannot be preserved for later analysis. A common testing error is introduced if the analyst does not obtain a representative sample. If sampling from a tap, let the water flow for at least five minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample container so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Avoid plastic containers since these may have a chlorine demand. Pre-treat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized water. If sample containers are rinsed thoroughly with deionized water after use, only occasional pretreatment is necessary. A pre-treated BOD bottle with a ground-glass stopper is an ideal sample container for chlorine analysis.

Treating Analysis Labware

Glassware used in this test must be chlorine demand-free. Fill the 100 mL mixing cylinder and sample container with a dilute solution of chlorine bleach prepared by adding 1 mL of commercial bleach to 1 liter of water. Soak in this solution at least one hour. After soaking, rinse thoroughly with deionized water and allow to dry before use. If the mixing cylinder is rinsed thoroughly with deionized water and allowed to dry after use, only occasional pretreatment is necessary. Do not use the same mixing cylinder for Free and Total Chlorine analysis.

Treat the Pour-Thru Cell similarly with dilute bleach and let stand for several minutes. Rinse several times with deionized water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods. Remove the buildup by rinsing the cell with 5.25 N sulfuric acid followed by several rinsings with deionized water.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the chlorine voluette ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* for more information.
4. Snap the top off a Chlorine Voluette® Ampule Standard Solution, 50 to 75-mg/L Cl_2.
5. Prepare three sample spikes. Use the TenSette® Pipet to add 0.3, 0.6, and 0.9 mL of standard to three 80-mL samples, respectively. Swirl gently to mix.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision

Standard: 1.07 mg/L Cl_2

Program	95% Confidence Limits of Distribution
82	1.06–1.08 mg/ L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.02 mg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chlorine can be present in water as free available chlorine and as combined available chlorine. Both forms can exist in the same water and can be determined together as the total available chlorine. Free chlorine is available as hypochlorous acid or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride, and other chloro derivatives.

The combined chlorine oxidizes iodide in the reagent to iodine. The iodine reacts with DPD (N,N-diethyl-p-phenylenediamine) indicator along with free chlorine present in the sample to form a red color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test. Subtract the results from the results of the total chlorine test to obtain combined chlorine. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Rapid Liquid Total Chlorine Reagent Set			25570-00
Includes:			
DPD Indicator Powder		24 g	22972-55
Total Chlorine Indicator Solution	1 mL	473 mL	22634-11
Total Chlorine Buffer Solution	1 mL	473 mL	22635-11

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cylinder, mixing, glass, 100-mL	1	each	1896-42
Dispenser, fixed volume, 1.0-mL RePipet Jr.	2	each	21113-01
Pour-Thru Cell Kit	1	each	59404-00

Required Standards

Chlorine Standard Solution, Voluette® Ampule, 50–75 mg/L, 10-mL		16/pkg	14268-10
Chlorine Standard Solution, Voluette® Ampule, 50–75 mg/L, 2-mL		20/pkg	14268-20
Water, deionized		4 L	272-56

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Chlorine, Total

★Method 8370

DPD Method*

Pour-Thru Cell

ULR (2 to 500 µg/L as Cl_2)

Scope and Application: For detecting trace levels of chlorine and chloramines in clean waters relatively free of color and turbidity; USEPA accepted for reporting for drinking water analysis

* U.S. Patent 5,362,650

Tips and Techniques

- Analyze samples immediately. Chlorine is not stable in an aqueous solution.
- A reagent blank value for a combined lot of indicator/buffer reagent solutions should be determined at least once a day. If sample color or turbidity fluctuates frequently during the day, determine a reagent blank for each sample. See *Determining the Reagent Blank Value on page 3*. To store the reagent blank value, see *Creating a User Program Based on a Hach Program* in the instrument manual.
- The ampules contain more than 1.0 mL of solution for ease of transfer. Discard excess reagent in the ampule.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

DR/2400 Pour-Thru Cell — Method 8370

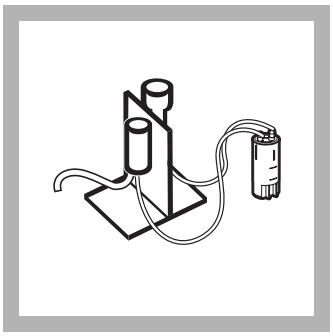
1. Touch

Hach Programs.

Select program

86 Chlor. Total ULR.

Touch **Start**.

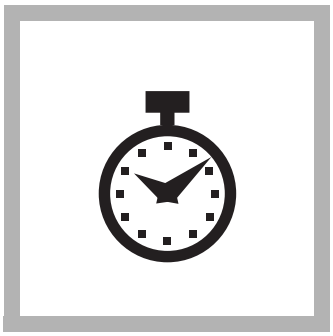


2. Install the Pour-Thru Cell and the multi-pathlength cell adapter in the sample cell compartment. Use the 25-mm cell pathlength.

Note: *See Section 2.6.1 on page 26 in the Instrument manual for installation details.*

Flush with 50-mL of deionized water.

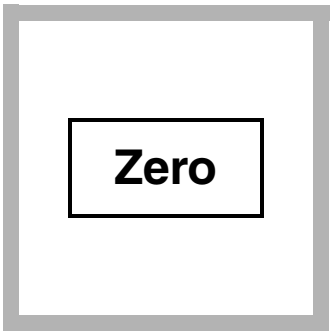
3. Pour at least 50 mL of sample into the Pour-Thru Cell.



4. After the flow stops, touch the timer icon.

Touch **OK**.

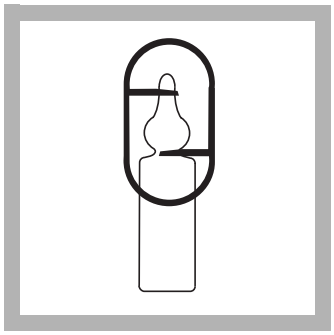
A three-minute reaction period will begin. This time allows turbidity or solids to settle and ensures a stable reading.



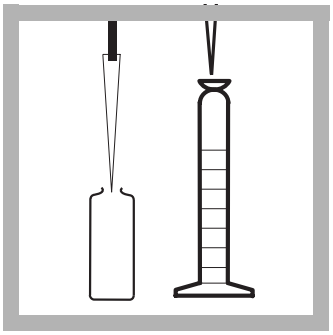
5. When the timer beeps, touch **Zero**.

The display will show:

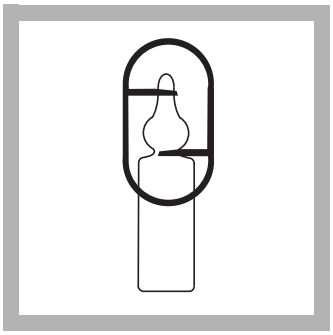
0 µg/L



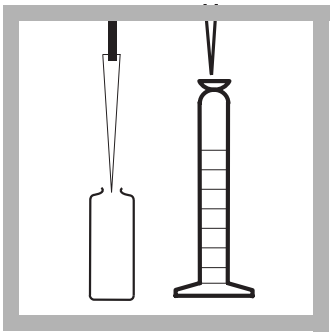
6. Break open one ULR Chlorine Buffer Solution Ampule.



7. Using a TenSette® Pipet and a clean tip, transfer 1.0 mL of buffer from the ampule to a clean, treated 50-mL graduated mixing cylinder.

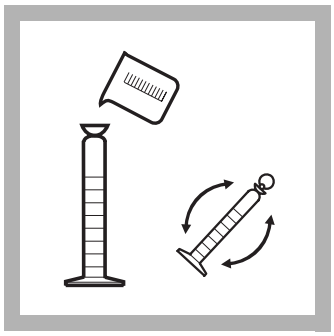


8. Break open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine.

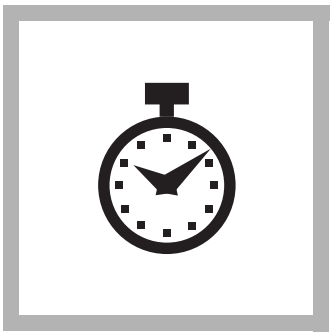


9. Using a TenSette® Pipet and a clean tip, transfer 1.0 mL of indicator from the ampule to the graduated mixing cylinder. Swirl to mix.

Proceed to step 10 within one minute.



10. Avoiding extra agitation, carefully fill the cylinder to the 50-mL mark with sample. Stopper the cylinder. Gently invert it twice to mix. This is the prepared sample.

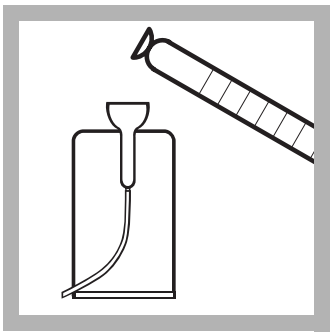


11. Touch the timer icon.

Touch **OK**.

A three-minute reaction time will begin.

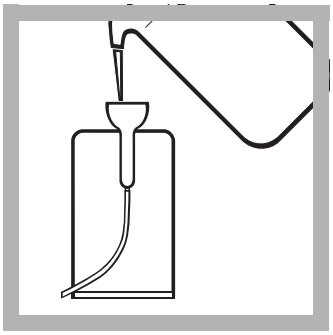
Measure the reacted sample 3–4 minutes after mixing the sample and reagents. If less than three minutes elapses, the reaction with chloramines may be incomplete. A reading after four minutes may result in higher reagent blank values.



12. Introduce the contents of the graduated mixing cylinder into the Pour-Thru cell.

When the timer beeps, touch **Read**. The result in µg/L chlorine will appear.

Note: *If a dechlorinating agent (e.g. sulfite or sulfur dioxide) is present, the sample result (corrected for the reagent blank) will read "0" or a slightly negative value.*



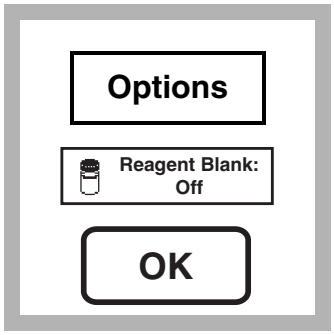
13. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Determining the Reagent Blank Value

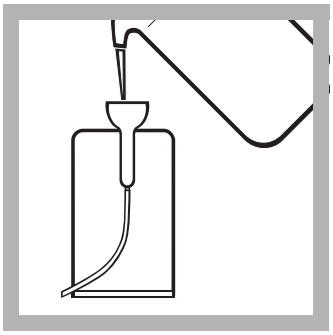
1. Touch **Hach Programs**.

Select program **86 Chlor. Total ULR**.

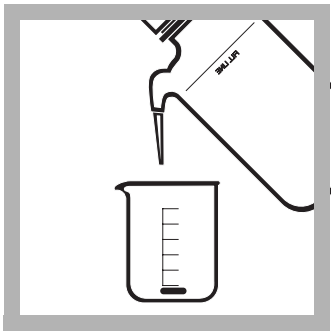
Touch **Start**.



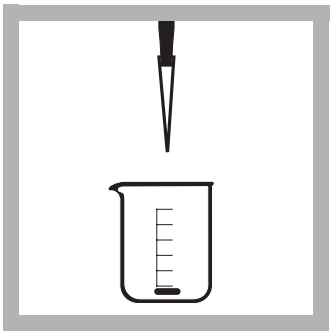
2. Make sure that the reagent blank setting is off. Touch **Options**, then **Reagent Blank**. Touch **Off**. Touch **OK** and **Return**.



3. Install the Pour-Thru Cell and cell adapter in the sample cell compartment. Flush with 50-mL of deionized water.

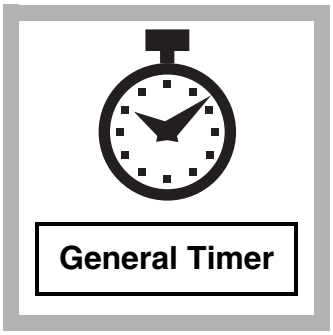


4. Collect about 100 mL of deionized or tap water in a clean, 250-mL beaker.



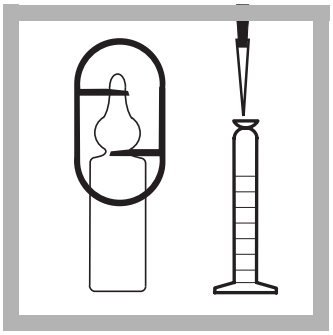
5. Using a TenSette® Pipet, add 1.0 mL of Blanking Reagent to the beaker. Swirl several times to mix.

The Blanking Reagent removes chlorine and chloramines from the water.

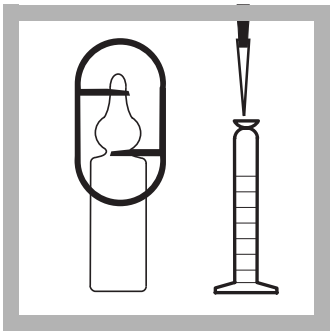


6. Touch the timer icon, then the **General Timer** option.

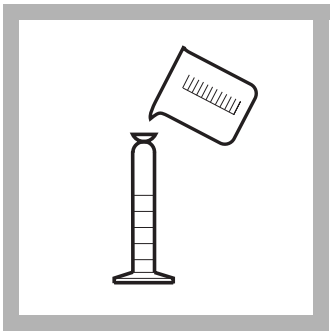
Set a five-minute timer. Touch **OK**. A five-minute dechorination period will begin.



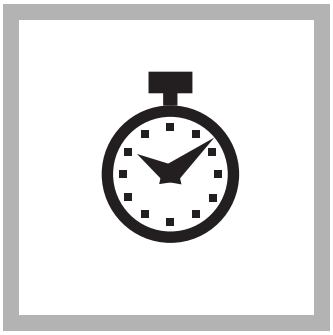
7. After the timer beeps, break open one ampule of ULR Chlorine Buffer Solution. Using a TenSette® Pipet and clean tip, transfer 1.0 mL of buffer from the ampule to a clean 50-mL mixing graduated cylinder.



8. Break open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine. Using a TenSette® Pipet and a clean tip, transfer 1.0 mL of indicator from the ampule to the cylinder. Swirl to mix the reagents. Proceed to step 9 within one minute.



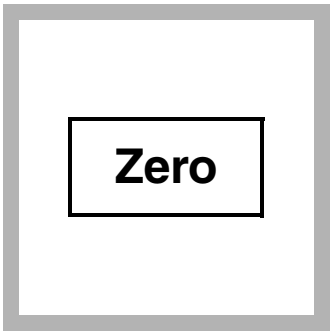
9. Fill the cylinder to the 50-mL mark with dechlorinated water from step 2. Cap and invert twice to mix. Save the remaining water for step 11.



10. Touch the timer icon. Touch the three-minute timer.

Touch **OK**. A three-minute reaction period will begin.

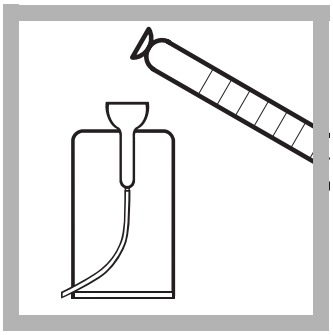
11. During the reaction period, flush the Pour-Thru Cell with the remainder of original dechlorinated water from step 9.



12. When the flow stops, touch **Zero**.

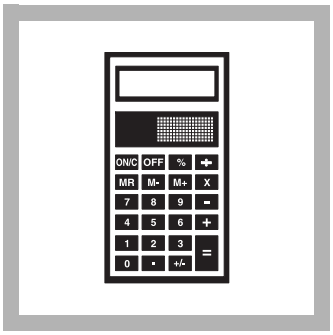
The display will show:

0 µg/L Cl_2



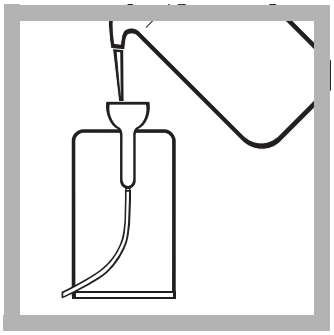
When the timer beeps, introduce the contents of the cylinder into the Pour-Thru Cell. When the flow stops, touch **Read**.

The result in µg/L chlorine will appear.



13. Use this value to correct the sample result obtained in this procedure.

Note: *See* Creating a User Program Based on a Hach Program *in the instrument manual for details on saving a reagent blank value.*



14. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Interfering Substance	Interference Levels and Treatments
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Copper, Cu^{2+}	Greater than 1000 µg/L
Iodine, I_2	Interferes at all levels
Iron (Fe^{3+})	Greater than 1000 µg/L
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 6 drops Potassium Iodide (Cat. No. 343-32) (30-g/L) to a 50-mL sample. **3.** Mix and wait 3 minutes. **4.** Add 6 drops Sodium Arsenite* (Cat. No. 1047-32) (5-g/L) and mix. **5.** Analyze the treated sample as described in the procedure. **6.** Subtract the result of this test from the original analysis to obtain the correct chlorine concentration.
Nitrite, NO_2^- (uncommon in clean waters)	See table below
Ozone, O_3	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7. See *Section 3.3 Interferences* on page *41*.

mg/L nitrite	Apparent µg/L chlorine
2.0 mg/L	3 µg/L
5.0 mg/L	5 µg/L
10.0 mg/L	7 µg/L
15.0 mg/L	16 µg/L
20.0 mg/L	18 µg/L

* Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See *Section 4* for more information on proper disposal of these materials.

Sample Collection, Storage, and Preservation

Analyze samples for chlorine immediately after collection. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (0.5 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

A common error in testing for chlorine is obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Treating Analysis Labware

Glassware used in this test must be chlorine demand-free. Treat all glassware with a dilute solution of chlorine bleach prepared by adding 0.5 mL of commercial bleach to 1 liter of water. Soak glassware in this solution at least on hour. After soaking, rinse the glassware with copious amounts of deionized water and allow to dry before use.

Treat the Pour-Thru Cell similarly with dilute bleach and let stand for several minutes and then rinse several times with deionized water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N Sulfuric Acid (Cat. No. 2449-53) followed by several rinsings with deionized water.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify that the units displayed are in mg/L.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the Chlorine Voluette® Ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a LR Chlorine Voluette Ampule Standard, 20–30 mg/L Cl_2.

5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-41) with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision
Standard: 290 µg/L Cl_2

Program	95% Confidence Limits of Distribution
86	287–293 µg/L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	2 µg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

This method is designed for clean water, low in color and turbidity. The main applications include monitoring for trace chlorine break-through of activated carbon beds and feedwater to reverse osmosis membranes or ion-exchange resins.

Several modifications to the normal DPD chlorine method are necessary to measure trace levels of chlorine. The Pour-Thru Cell must be used in the spectrophotometer. Liquid reagents are also required. The reproducible optics of the Pour-Thru Cell give more stable readings than is possible with movable sample cells, resulting in more stable measurements.

The reagents are packaged in ampules and sealed under argon gas to ensure stability. Use of liquid reagents eliminates any slight turbidity that might be caused by using powdered reagents. Due to the possible oxidation of the reagents (which could give a positive chlorine reading in the blank), a reagent blank must be determined at least once a day for each lot of reagent used. This reagent blank value is subtracted from the sample result and the corrected value is the actual chlorine concentration. Test results are measured at 515 nm.

Chlorine, Total

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No
ULR Chlorine Reagent Set (about 20 tests)			25630-00
Includes:			
ULR Chlorine Buffer Solution, 1.5-mL ampules	1 mL	20/pkg	24931-20
DPD Indicator Solution for ULR Chlorine, 1.5-mL ampules	1 mL	20/pkg	24932-20
Blanking Reagent for ULR Chlorine	1 mL	29 mL	24930-23

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Beaker, 250-mL	1	each	500-46H
Cylinder, mixing, graduated, 50-mL	1	each	1896-41
Pour-Thru Cell Kit	1	each	59404-00
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96

Required Standards

Chlorine Standard Solution, 2-mL Voluette® Ampules, 20–30 mg/L		20/pkg	26300-20

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WORLD HEADQUARTERS
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FAX: (970) 669-2932

3/04 1ed

Chlorine, Total

★**Method 10014**

DPD Method*

Pour-Thru Cell

ULR (2 to 500 µg/L as Cl_2)

Scope and Application: For testing trace levels of chlorine and chloramines in treated domestic and industrial wastewater; USEPA accepted for reporting for wastewater analysis**

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

** U.S. Patent 5,362,650 covers the procedure. U.S. Patent 5,549,816 covers the OriFlo™ Filtration System.

Tips and Techniques

- Analyze samples immediately. Chlorine is not stable in an aqueous solution.
- A reagent blank value for a combined lot of indicator/buffer reagent solutions should be determined at least once a day. See *Determining the Reagent Blank Value on page 4*. To store the reagent blank value, see *Creating a User Program Based on a Hach Program* in the instrument manual.
- The reagent blank value is normally less than 5 µg/L. If the value is greater than 5 µg/L, an interfering substance may be present in the blanking water or the DPD Indicator may be degrading. If there is doubt about the reagents, repeat the reagent blank determination using chlorine-demand-free water for the sample. Blanks up to 5 µg/L may be used.
- Use a new filter for each test. Using an unspecified filter may give low analysis results or inability to filter the required volume.
- The ampules contain more than 1.0 mL of solution for ease of transfer. Discard excess reagent in the ampule.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

DR/2400 Pour-Thru Cell — Method 10014

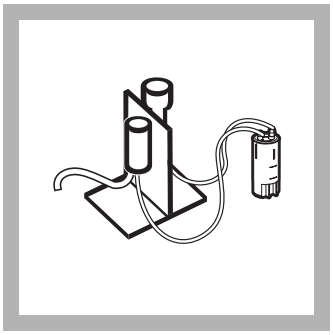
1. Touch

Hach Programs.

Select program

86 Chlor. Total ULR.

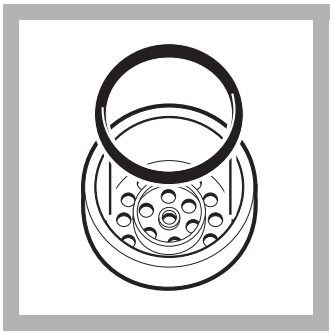
Touch **Start**.



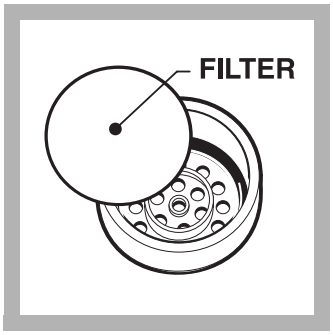
2. Install the Pour-Thru Cell and multi-pathlength cell adapter in the sample cell compartment. Use the 25-mm cell pathlength.

Note: *See Section 2.6.1 on page 26 in the Instrument manual for installation details.*

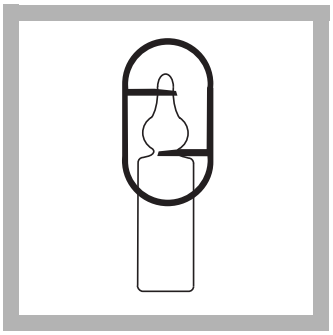
Flush with 50-mL of deionized water.



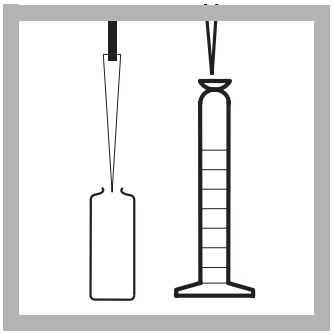
3. Unscrew the cap from the OriFlo™ plunger assembly. Be sure that the O-ring is properly seated in the cap.



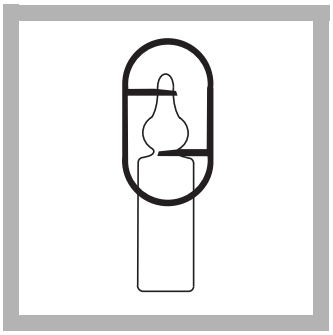
4. Install a new, 3-micron filter into the cap well. Wet the filter with a few drops of deionized water. Reassemble and hand-tighten the cap onto the plunger.



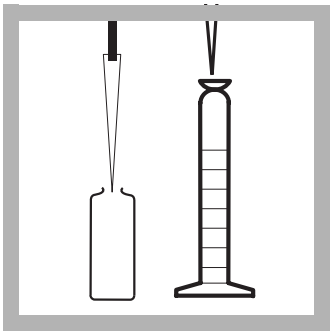
5. Break open one ULR Chlorine Buffer Solution Ampule.



6. Using a TenSette® Pipet and a clean tip, transfer 1.0 mL of buffer from the ampule to a clean, treated 50-mL graduated mixing cylinder.

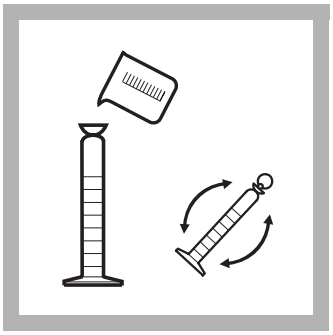


7. Break open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine.

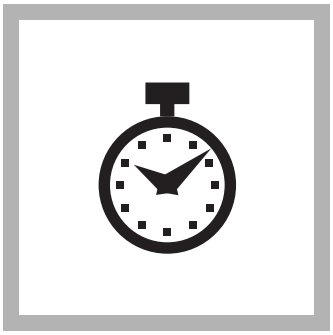


8. Using a TenSette® Pipet and a clean tip, transfer 1.0 mL of indicator from the ampule to the graduated mixing cylinder. Swirl to mix.

Proceed to step 9 within one minute.



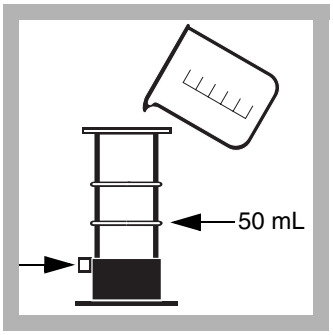
9. Avoiding extra agitation, carefully fill the cylinder to the 50-mL mark with sample. Stopper the cylinder. Gently invert it twice to mix. This is the prepared sample.



10. Touch the timer icon. Touch **OK**.

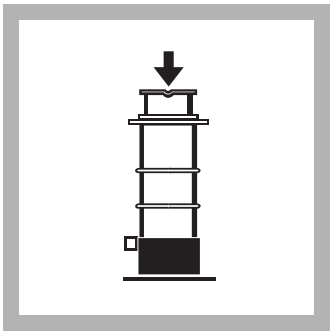
A three-minute reaction time will begin. Perform steps 11–16 during this period.

Measure the reacted sample 3–6 minutes after mixing the sample and reagents. If less than three minutes elapses, the reaction with chloramines may be incomplete. A reading after six minutes may result in higher reagent blank values.

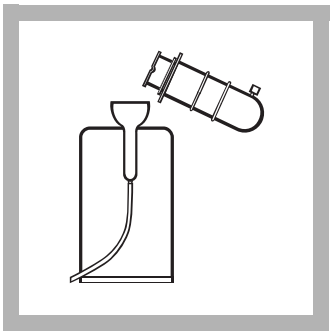


11. Push the valve button the OriFlo™ barrel assembly to the "closed" position. Place the barrel assembly into its stand. Pour approximately 50 mL of the original sample into the barrel.

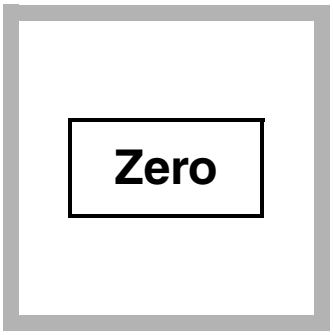
The lower ring on the barrel assembly represents about a 50-mL volume.



12. Insert the plunger into the barrel and slowly push the plunger down with even pressure, until the plunger is fully seated.



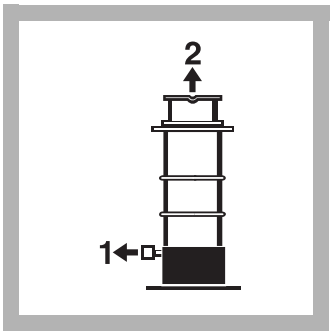
13. Pour the filtered sample from the plunger reservoir into the Pour-Thru Cell.



14. After the flow stops, touch **Zero**.

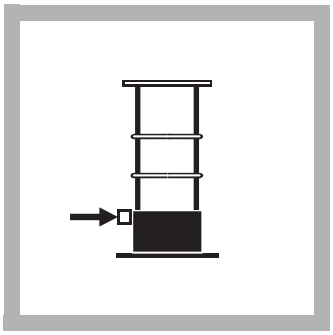
The display will show:

0 µg/L Cl_2

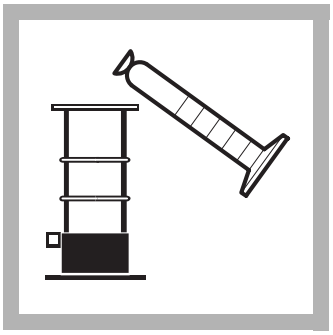


15. Pull the barrel's valve button out to the "open" position. Pull the plunger up to separate it from the barrel assembly. Discard the remaining unfiltered sample.

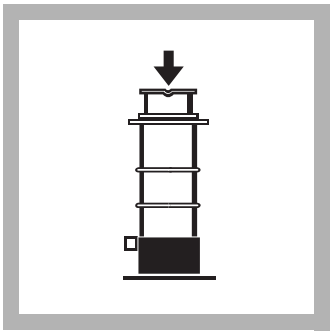
Note: *You may need a new membrane for very turbid samples. Alternatively, use a second Quick Filter unit with a new membrane filter installed.*



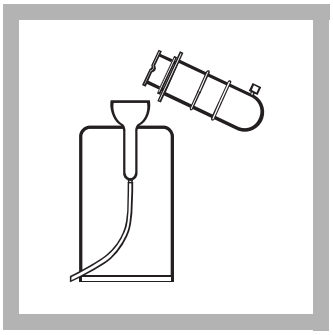
16. Push the barrel's valve button to the "closed" position. Place the barrel assembly into its stand.



17. When the timer beeps, pour the contents of the mixing cylinder into the barrel.



18. Insert the plunger into the barrel and slowly push the plunger down with even pressure, until the plunger is fully seated.

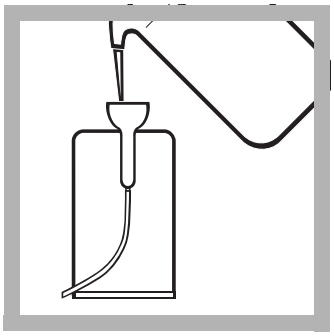


19. Pour the filtered, reacted sample from the plunger reservoir into the Pour-Thru Cell.

When the flow stops, touch **Read**.

Results in µg/L chlorine will appear.

Note: *If a dechlorinating agent (e.g., sulfite or sulfur dioxide) is present, the sample result, corrected for the reagent blank, will read "0" or a slightly negative value.*



20. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

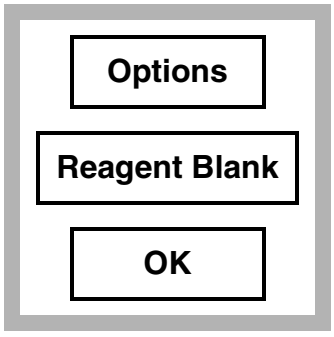
21. Determine a reagent blank using the procedure following these steps.

Determining the Reagent Blank Value

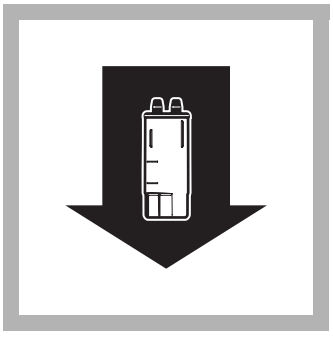
1. Touch **Hach Programs**.

Select program **86 Chlor. Total ULR**.

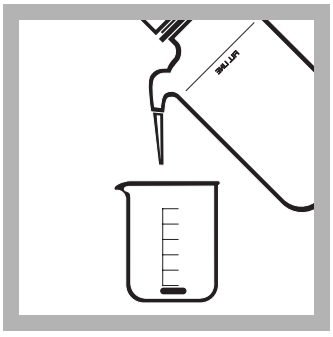
Touch **Start**.



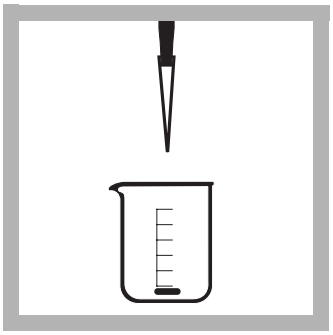
2. Make sure that the reagent blank setting is off. Touch **Options**, then **Reagent Blank**. Touch **Off**. Touch **OK** and **Return**.



3. Install the Pour-Thru Cell and multi-pathlength cell adapter in the sample cell compartment. Flush with 50-mL of deionized water.

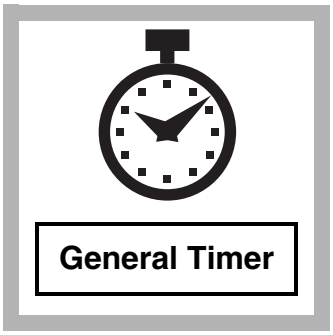


4. Collect about 100 mL of deionized or tap water in a clean, 250-mL beaker.



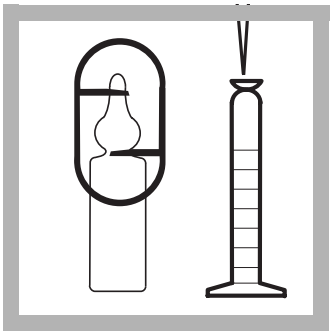
5. Using a TenSette® Pipet, add 1.0 mL of Blanking Reagent to the beaker. Swirl several times to mix.

The Blanking Reagent removes chlorine and chloramines from the water.

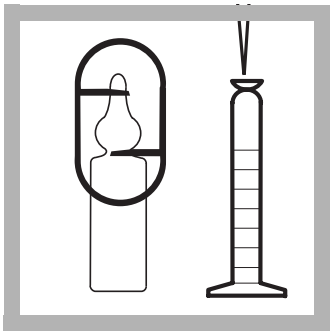


6. Touch the timer icon, then the **General Timer** option.

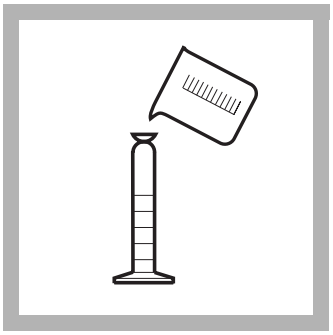
Set a five-minute timer. Touch **OK**. A five-minute dechlorination period will begin.



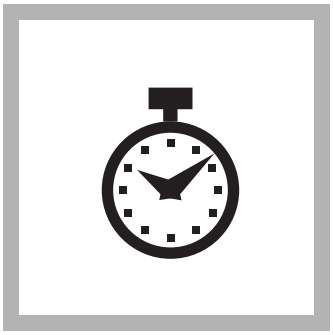
7. After the timer beeps, break open one ampule of ULR Chlorine Buffer Solution. Using a TenSette® Pipet and clean tip, transfer 1.0 mL of buffer from the ampule to a clean 50-mL mixing graduated cylinder.



8. Break open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine. Using a TenSette® Pipet and a clean tip, transfer 1.0 mL of indicator from the ampule to the cylinder. Swirl to mix the reagents. Proceed to step 9 within one minute.



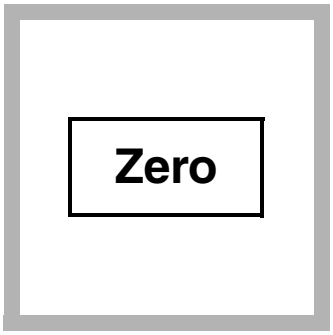
9. Fill the cylinder to the 50-mL mark with dechlorinated water from step 5. Cap and invert twice to mix. Save the remaining water for step 11.



10. Touch the timer icon. Touch the three-minute timer.

Touch **OK**. A three-minute reaction period will begin.

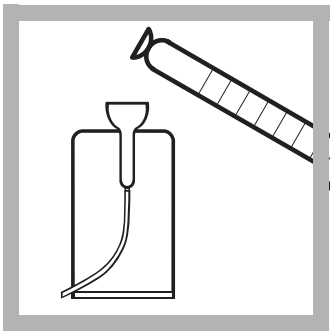
11. During the reaction period, flush the Pour-Thru Cell with the remainder of original dechlorinated water from step 9.



12. When the flow stops, touch **Zero**.

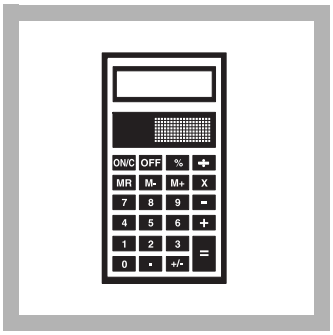
The display will show:

0 µg/L Cl_2

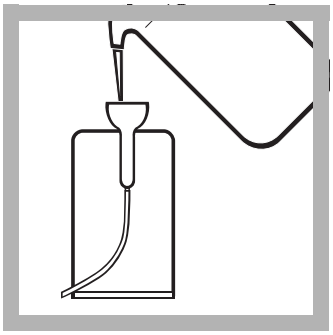


13. When the timer beeps, introduce the contents of the cylinder into the Pour-Thru Cell.

When the flow stops, touch **Read**. Results will appear in µg/L Cl_2.



14. Use this value to correct the sample result obtained in this procedure.



15. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Interfering Substance	Interference Levels and Treatments
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere
Copper, Cu^{2+}	Greater than 1000 µg/L
Iodine, I_2	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 6 drops Potassium Iodide (Cat. No. 343-32) (30-g/L) to a 50-mL sample. **3.** Mix and wait 3 minutes. **4.** Add 6 drops Sodium Arsenite* (Cat. No. 1047-32) (5-g/L) and mix. **5.** Analyze the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Nitrite, NO_2^-	mg/L nitrite / Apparent µg/L chlorine 2.0 mg/L / 3 µg/L 5.0 mg/L / 5 µg/L 10.0 mg/L / 7 µg/L 15.0 mg/L / 16 µg/L 20.0 mg/L / 18 µg/L
Ozone, O_3	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7. See *Section 3.3 Interferences* on page *41*.

* Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See *Section 4* for more information on proper disposal of these materials.

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no head space (air) above the sample. Perform the chlorine analysis immediately.

Treating Analysis Labware

Glassware used in this test must be chlorine demand-free. Treat all glassware with a dilute solution of chlorine bleach prepared by adding 0.5 mL of commercial bleach to 1 liter of water. Soak glassware in this solution at least one hour. After soaking, rinse the glassware thoroughly with deionized water and allow to dry before use.

Treat the Pour-Thru Cell similarly with dilute bleach and let stand for several minutes and then rinse several times with deionized water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to remain in the cell for long periods after measurement. Remove the buildup by rinsing the cell with 5.25 N Sulfuric Acid (Cat. No. 2449-53) followed by several rinsings with deionized water.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the Chlorine Voluette® Ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a LR Chlorine Voluette® Ampule Standard, 20–30 mg/L Cl_2.

5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-41) with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision
Standard: 290 µg/L Cl_2

Program	95% Confidence Limits of Distribution
86	287–293 µg/L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	2 µg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

It is essential that interfering sample turbidity is removed using a 3-micron membrane filter. To avoid chlorine loss, the filtration is done after reacting the DPD with the chlorine in the sample. The filter used has been specifically selected to avoid retention of the colored product. Sample color is compensated by zeroing the spectrophotometer on a filtered sample.

Several modifications to the normal DPD chlorine method are necessary to measure trace levels of chlorine. The Pour-Thru Cell must be used in the spectrophotometer. Liquid reagents are also required. The reproducible optics of the Pour-Thru Cell give more stable readings than is possible with movable sample cells, resulting in more stable measurements.

The reagents are packaged in ampules and sealed under argon gas to ensure stability. Use of liquid reagents eliminates any slight turbidity that might be caused by using powdered reagents. Due to the possible oxidation of the reagents (which could give a positive chlorine reading in the blank), a reagent blank must be determined at least once a day for each lot of reagent used. This reagent blank value is subtracted from the sample result and the corrected value is the actual chlorine concentration. Test results are measured at 515 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No
ULR Chlorine Reagent Set (about 20 tests)			25630-00
Includes:			
ULR Chlorine Buffer Solution, 1.5 mL ampules	1 mL	20/pkg	24931-20
DPD Indicator Solution for ULR Chlorine, 1.5 mL ampules	1 mL	20/pkg	24932-20
Blanking Reagent for ULR Chlorine	1 mL	29 mL	24930-23

Required Apparatus

ULR Chlorine Apparatus Set			25956-00
Includes:			
Membrane filters, 3 micron, 25-mm	1	25/pkg	25940-25
Oriflo Assembly	1	each	49660-00
Adapter, multi-pathlength cell	1	each	59466-00
Beaker, 250-mL	1	each	500-46H
Cylinder, mixing, graduated, 50-mL	1	each	1896-41
Pour-Thru Cell Kit	1	each	59404-00
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96

Required Standards

Chlorine Standard Solution, 2-mL Voluette® Ampules, 20–30 mg/L		20/pkg	26300-20

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Chlorine, Total

Method 10101 **DPD Method***

Test 'N Tube™ Vials **(0.09 to 5.00 mg/L)**

Scope and Application: For testing higher levels of total (free plus combined) chlorine in drinking water, treated wastewater, cooling water, or industrial process water

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- Analyze samples immediately. Do not preserve samples for later analysis.
- After adding sample to the Test 'N Tube™, a pink color will develop if total chlorine is present.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- For chloramination disinfection control, use Method 10172, Chloramine (Mono), High Range.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Test 'N Tube Method 10101

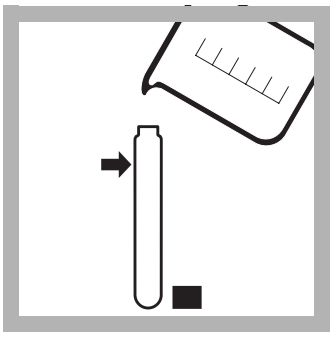
1. Touch

Hach Programs.

Select program

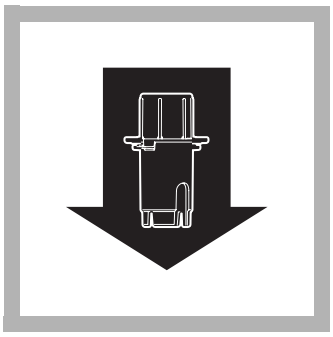
89 Chlor. F&T HR TNT.

Touch **Start**.



2. Fill an empty Test 'N Tube™ vial to the top of the Hach label with sample. (This is the blank.)

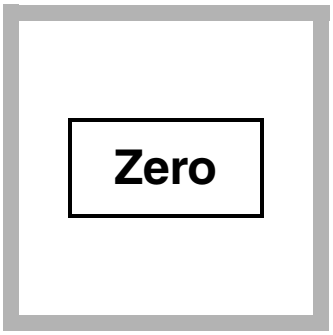
3. Wipe the outside of the vial with a damp towel, then a dry towel to remove fingerprints and other marks.



4. Install the 16-mm adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for installation details.*

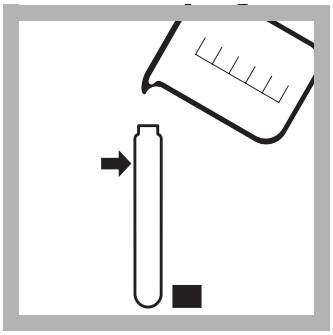
Insert the blank into the adapter.



5. Touch **Zero**.

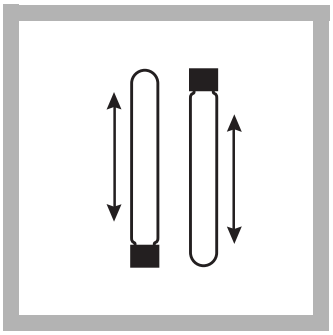
The display will show:

0.00 mg/L Cl_2

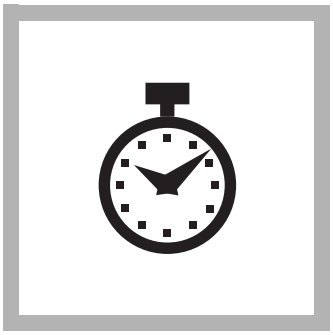


6. Remove the cap from a Free Chlorine DPD Test 'N Tube™. Add 10 mL of sample to the tube. (Fill the vial to the top of the Hach label.)

This is the prepared sample.



7. Cap and slowly invert at least 10 times to dissolve the powder. (Ten inversions should take at least 30 seconds. One inversion equals turning the vial upside down, then returning it to an upright position.)

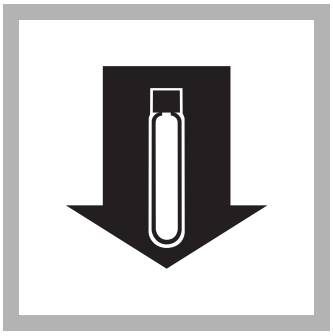


8. Touch the timer icon.

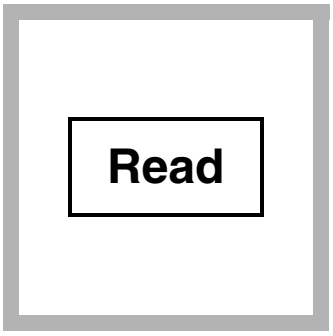
Touch **OK**.

A two-minute reaction period will begin.

9. When the timer beeps, wipe the outside of the vial that contains the prepared sample.



10. Place the sample in the adapter.



11. Touch **Read**.

Results will appear in mg/L Cl_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with1 N Sodium Hydroxide. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *3.1.3 Correcting for Volume Additions*).
Alkalinity	Greater than 300 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (see *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide, ClO_2	Interferes at all levels
Chloramines, organic	May interfere

(continued)

Interfering Substance	Interference Levels and Treatments
Hardness	No effect at less than 1,000 mg/L as $CaCO_3$
Iodine, I_2	Interferes at all levels
Manganese, oxidized (Mn^{4+}, Mn^{7+}) or Chromium, oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide (30-g/L) (Cat. No. 343-32) to a 25-mL sample. **3.** Mix and wait 1 minute. **4.** Add 3 drops Sodium Arsenite* (5-g/L) (Cat. No. 1047-32) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result of this test from the original analysis to obtain the correct chlorine concentration.
Ozone, O_3	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid, 1.000 N, Cat. No. 1270-32) or base (Sodium Hydroxide, 1.00 N, Cat. No. 1045-32).

* Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See *Section 4* for more information on proper disposal of these materials.

Sample Collection, Storage and Preservation

Analyze samples for chlorine immediately after collection. Free chlorine and combined chlorine are strong oxidizing agents and are unstable in natural waters. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of free chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

A common error in testing for chlorine is obtaining an unrepresentative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. Perform the chlorine analysis immediately.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A keypad will appear. Enter the average chlorine concentration shown on the certificate enclosed with the Chlorine Voluette® Ampules. Touch **OK**.
3. A summary of the Standard Additions procedure will appear. Touch **OK** to accept the values for standard concentration, sample volume, and spike volumes as shown. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.

4. Snap the neck off a HR Chlorine PourRite® Ampule Standard, 50-75 mg/L Cl_2.
5. Use the TenSette® Pipet to add 0.1 mL of standard to a 10-mL sample and mix thoroughly.
6. Analyze the standard addition sample as described in the procedure above. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision

Standard: 2.33 mg/L Cl_2

Program	95% Confidence Limits of Distribution
89	2.27–2.39 mg/L Cl_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.09 mg/L Cl_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can exist in the same water and be determined together as the total chlorine. Free chlorine is present as hypochlorous acid or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives.

Free or combined chlorine oxidizes iodide in the reagent to iodine. The iodine and chlorine react with DPD (N,N-diethyl-p-phenylenediamine) to form a magenta color, which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Test 'N Tube™ DPD Total Chlorine Reagent	1 vial	50/pkg	21056-45

Required Standards

Chlorine Standard Solution, 2-mL PourRite® Ampule, 50–75 mg/L		20/pkg	14268-20

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Chromium, Hexavalent

★Method 8023 — 1,5-Diphenylcarbohydrazide Method*

Powder Pillows or AccuVac® Ampuls — (0.01 to 0.70 mg/L Cr^{6+})

Scope and Application: For water and wastewater;
USEPA accepted for reporting for wastewater analysis**

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Procedure is equivalent to USGS method 1-1230-85 for wastewater.

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. See the instrument manual for more information on *Running a Reagent Blank*.
- At high chromium levels, a precipitate will form. Dilute the sample according to *Section 2.7 Sample Dilution* on page *21*.
- The final samples are highly acidic. Neutralize to pH 6–9 with Sodium Hydroxide Standard Solution (Cat. No. 2450-26) and flush down the drain for disposal. For more information on pollution prevention and waste management, refer to *Section 4*.

Powder Pillows — Method 8023

1. Touch

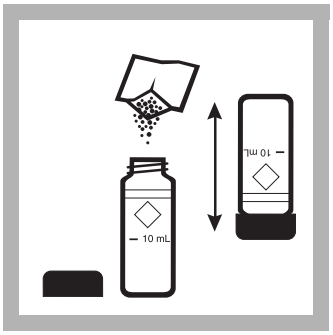
Hach Programs.

Select program

90 Chromium, Hex.

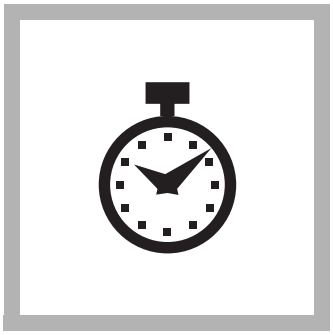
Touch **Start**.

2. Fill a round sample cell with 10 mL of sample.



3. Add the contents of one ChromaVer® 3 Reagent Powder Pillow to the sample cell (the prepared sample). Cap and invert gently to mix.

A purple color will form if hexavalent chromium is present.



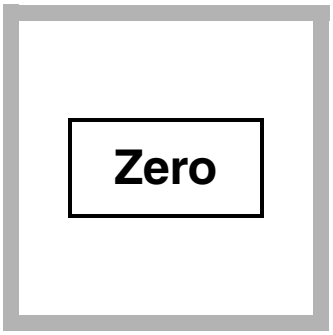
4. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

5. Fill another round sample cell with 10 mL of sample (the blank).

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L Cr^{6+}

8. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L Cr^{6+}.

AccuVac Ampul — Method 8023

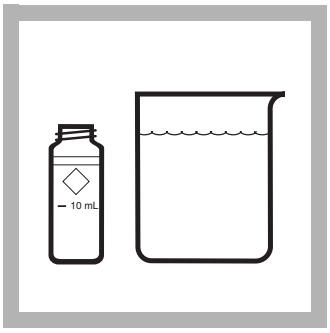
1. Touch

Hach Programs.

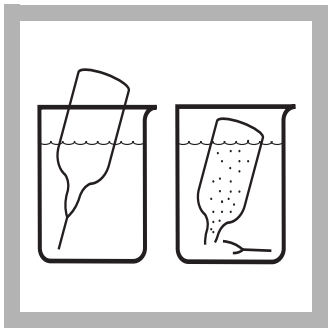
Select program

95 Chromium, Hex. AV.

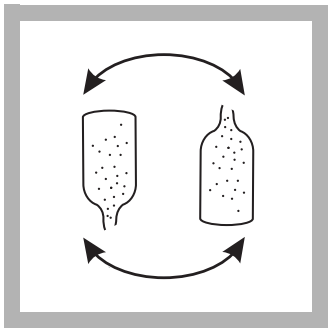
Touch **Start**.



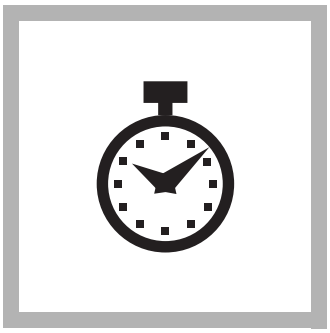
2. Fill a round sample cell with 10-mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.



3. Fill a ChromaVer 3 Reagent AccuVac® Ampul with sample (the prepared sample). Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampule several times to mix. Wipe off any liquid or fingerprints.

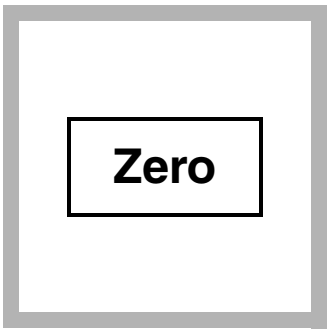


5. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L Cr6+

8. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L Cr^{6+}.

Interferences

Interfering Substance	Interference Levels and Treatments
Iron	May interfere above 1 mg/L
Mercurous & Mercuric Ions	Interfere slightly
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. See *Section 3.3 Interferences* on page *41*.
Vanadium	May interfere above 1 mg/L. Allow 10 minutes for the reaction period before reading.
Turbidity	For turbid samples, treat the blank with the contents of one Acid Reagent Powder Pillow (Cat. No. 2126-99). This will ensure that any turbidity dissolved by the acid in the ChromaVer 3 Chromium Reagent will also be dissolved in the blank.

Sample Collection, Preservation, and Storage

Collect samples in a cleaned glass or plastic container. Store at 4 °C (39 °F) up to 24 hours. Samples must be analyzed within 24 hours.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell, or AccuVac Ampul (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Chromium Voluette® Ampule Standard, 12.5 mg/L Cr^{6+}.

5. For analysis using powder pillows, use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively to three 25-mL samples and mix thoroughly. Transfer 10 mL of each solution into a 10-mL sample cell and analyze as described above.

Note: *For AccuVac ampules, fill three mixing cylinders (Cat. No. 1896-41) with 50-mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers. Analyze each standard addition sample as described in the procedure above.*

6. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 0.25-mg/L Cr^{6+} standard solution daily, as follows:

1. Using a 5.00 mL pipet (Cat. No. 14515-37) transfer 5.00 mL of Hexavalent Chromium Standard Solution, 50 mg/L, into a Class A 1000-mL volumetric flask (Cat. No. 14574-53).
2. Dilute to the mark with deionized water.
3. To adjust the calibration curve using the reading obtained with the 0.25-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Perform the hexavalent chromium procedure as described above. See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.25 mg/L Cr^{6+}

Program	95% Confidence Limits of Distribution
90	0.24–0.26 mg/L Cr^{6+}
95	0.24–0.26 mg/L Cr^{6+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
90	Entire range	0.010	0.01 mg/L Cr^{6+}
95	Entire range	0.010	0.01 mg/L Cr^{6+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Hexavalent chromium is determined by the 1,5-Diphenylcarbohydrazide method using a single dry powder formulation called ChromaVer 3 Chromium Reagent. This reagent contains an acidic buffer combined with 1,5-Diphenylcarbohydrazide, which reacts to give a purple color when hexavalent chromium is present. Test results are measured at 540 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
ChromaVer® 3 Chromium Reagent Powder Pillows	1 pillow	100/pkg	12710-99
or			
ChromaVer® 3 AccuVac® Ampuls	1 ampule	25/pkg	25050-25
Water, deionized		4 liters	272-56

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Beaker, 50-mL	1	each	500-41H

Required Standards

Chromium, Hexavalent Standard Solution, 10-mL Voluette® Ampules, 12.5-mg/L Cr^{6+}		16/pkg	14256-10
Chromium, Hexavalent Standard Solution, Chromium, Standard Solution, 50.0-mg/L Cr^{6+}		100 mL	810-42H

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In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Chromium, Hexavalent

1,5-Diphenylcarbohydrazide Method

UniCell™ Vials

(0.03 to 1.00 mg/L Cr^{6+})

Scope and Application: For wastewater process control

Tips and Techniques

- Undissolved chromium can only be determined after digestion with the Metal Prep Set (HCT 200).
- Adjust pH of preserved samples to between pH 3–9 before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check*.
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is between 15–25 °C
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

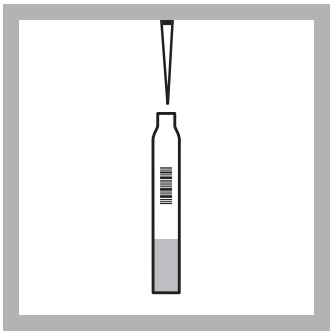
1. Touch

Hach Programs.

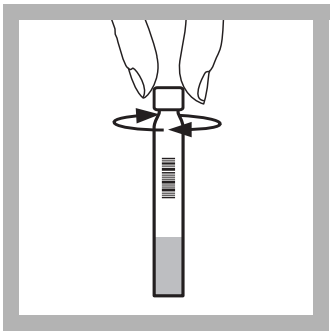
Select program

803 Chromium, HCT 156

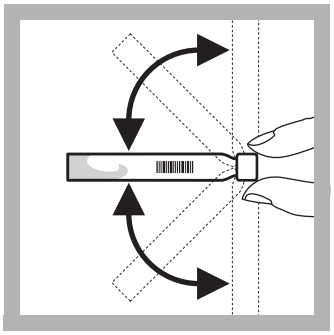
Touch **Start**.



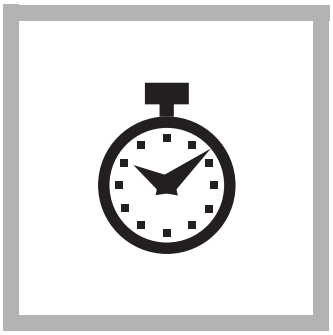
2. Pipet 4.0 mL of sample into a sample vial.



3. Cap the sample vial with **orange** UniCap B (HCT 156 B).



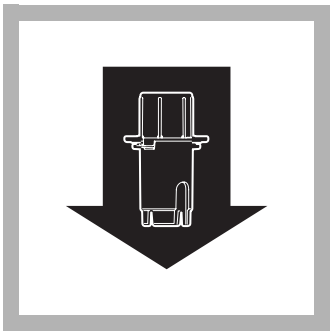
4. Invert the sample vial several times to mix.



5. Touch the timer icon.

Touch **OK**.

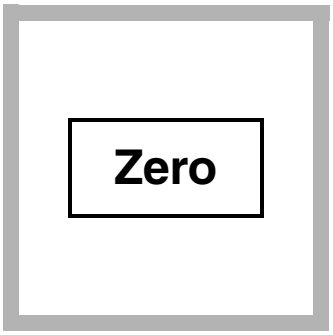
A five-minute reaction period will begin.



6. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

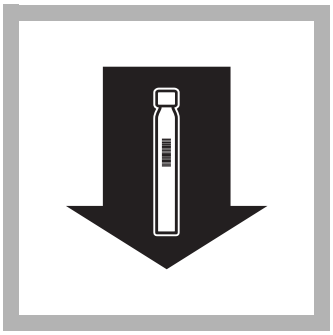
When the timer beeps, wipe the zero vial (**white** cap) and place it into the cell adapter.



7. Touch **Zero**.

The display will show:

0.00 mg/L Cr^{6+}
Underrange



8. Wipe the sample vial and place it into the cell adapter.

Touch **Read**.

Results will appear in mg/L Cr^{6+}.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Cl^-	1000 mg/L
Ca^{2+}	125 mg/L
Mg^{2+}, NH_4^+	100 mg/L
Zn^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}	50 mg/L
Cu^{2+}, Fe^{3+}	10 mg/L
Sn^{2+}	1 mg/L

Higher amounts of iron, copper, and reducing or oxidizing agents will cause a negative interference. Lead, mercury, and tin will cause a positive interference.

Note: *Chromium concentrations above 20 mg/L produce result displayed within the measuring ranges given above. In such cases it is recommended that an accuracy check be carried out by dilution.*

Note: *Undissolved chromium is not determined with the determination of chromium VI. Total Chromium can only be determined using the Total Chromium procedure.*

Sample Collection, Preservation, and Storage

Collect samples in a cleaned glass or plastic container. Store at 4 °C (39 °F) up to 24 hours. Samples must be analyzed within 24 hours.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Pipet 0.1, 0.2, and 0.3 mL of 50-mg/L Cr^{6+} standard solution, respectively, to three 25-mL samples and mix thoroughly.
5. Transfer 4 mL of each solution to three sample vials and analyze as described in the procedure. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 0.70-mg/L Cr^{6+} Standard solution daily, as follows:

1. Using a pipet, transfer 1.40 mL of Hexavalent Chromium Standard Solution, 50-mg/L, into a Class A 100-mL volumetric flask.
2. Dilute to the mark with deionized water.
3. To adjust the calibration curve using the reading obtained with the 0.70-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Perform the hexavalent chromium procedure as described above. See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 0.50 mg/L Cr^{6+}

Program	95% Confidence Limits of Distribution
803	0.38–0.62 mg/L Cr^{6+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
803	Entire range	0.010	0.01 mg/L Cr^{6+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chromium (VI) ions react with 1,5-diphenylcarbazide to 1,5-di-phenylcarbazone and Chromium (III), resulting in a red complex. Measurements are taken at 543 nm.

Required Reagents

Description	Unit	Cat. No.
Chromium - Cr(VI), UniCell™ HCT 156	23/pkg	HCT 156

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Flask, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 100-mL	each	20886-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pk/100	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	pk/400	27950-00
pH Paper	pk/100	26013-00

Optional Reagents

Chromium Standard Solution, 50-mg/L	100 mL	810-42 H
Metal Prep Set, HCT 200	50 digestions	HCT 200

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Chromium, Total

Method 8024 — **Alkaline Hypobromite Oxidation Method* ****

Powder Pillows — **(0.01 to 0.70 mg/L)**

Scope and Application: For water and wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** This procedure is equivalent to Standard Method 3500-CRD for wastewater.

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Prepare a boiling water bath for step 4.
- Use finger cots (Cat. No. 14647-02) to handle hot sample cells.
- Undissolved powder does not affect accuracy.

Powder Pillows — Method 8024

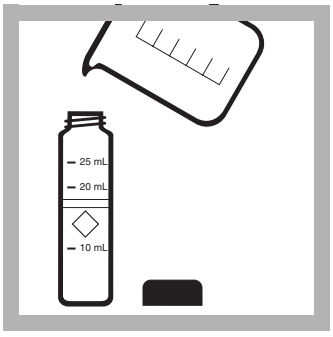
1. Touch

Hach Programs.

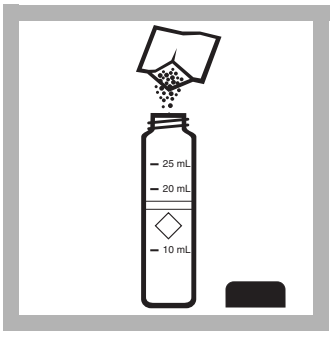
Select program

100 Chromium, Total.

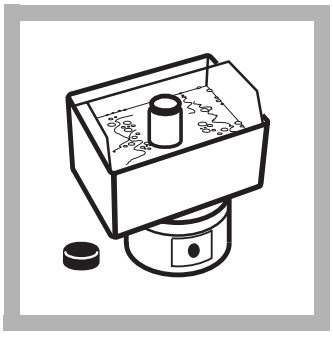
Touch **Start**.



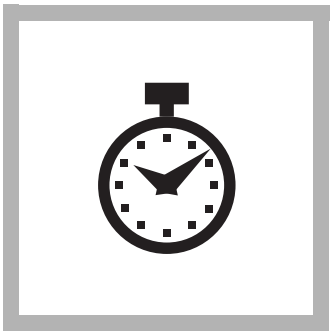
2. Fill a round sample cell with 25 mL of sample.



3. Add the contents of one Chromium 1 Reagent Powder Pillow (the prepared sample). Cap and invert to mix.



4. Remove the cap and place the prepared sample into a boiling water bath.

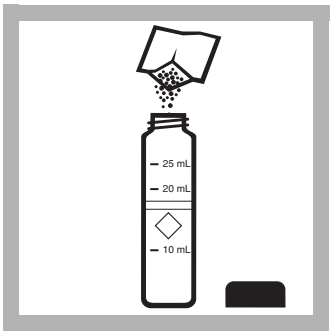


5. Touch the timer icon.

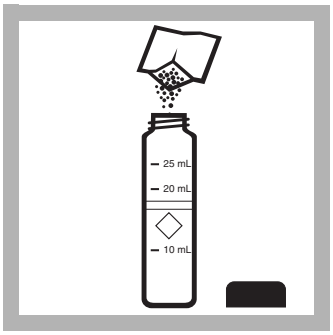
Touch **OK**.

A five-minute reaction period will begin.

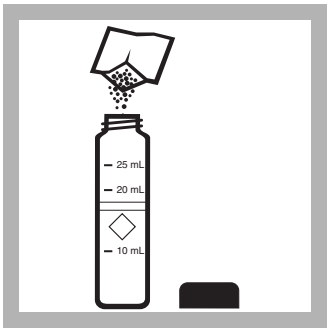
6. When the timer beeps, remove the prepared sample. Using running water, cool the cell to 25 °C. Be sure the caps are on tightly.



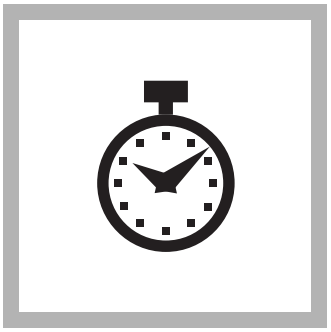
7. Remove cap and add the contents of one Chromium 2 Reagent Powder Pillow. Cap and invert to mix.



8. Remove cap and add the contents of one Acid Reagent Powder Pillow. Cap and invert to mix.



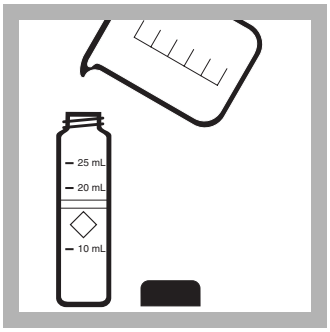
9. Remove cap and add the contents of one ChromaVer 3 Chromium Reagent Powder Pillow. Cap and invert to mix.



10. Touch the timer icon.

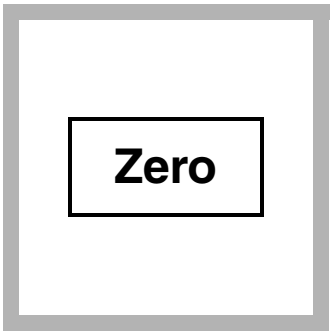
Touch **OK**.

A five-minute reaction period will begin.



11. When the timer beeps, fill another sample cell with 25 mL of sample (the blank).

12. Place the blank into the cell holder.

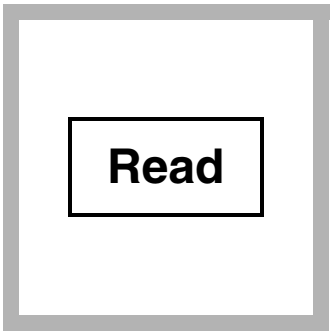


13. Touch **Zero**.

The display will show:

0.00 mg/L Cr

14. Place the prepared sample into the cell holder.



15. Touch **Read**.

Results will appear in mg/L Cr.

Interferences

Interfering Substance	Interference Levels and Treatments
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see *Section 3.3 Interferences* on page *41*.
Organic material	May inhibit complete oxidation of trivalent chromium. If high levels of organic material are present, see *Section 4* for instructions on sample digestion. Perform the analysis as described on the digested sample.
Turbidity	For turbid samples, treat the 25-mL blank and the sample the same during steps 2–8.

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic containers. To preserve samples, adjust the pH to 2 or less with nitric acid (Cat. No. 152-49). This requires approximately 2 mL per liter of the acid. Store preserved samples at room temperature up to six months. Adjust the pH to about 4 with 5.0 N Sodium Hydroxide (Cat. No. 2450-26) before analysis. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Trivalent Chromium Voluette® Ampule Standard, 12.5-mg/L as Cr^{3+}.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 0.25-mg/L trivalent chromium standard as follows:

1. Dilute 5.00 mL of Trivalent Chromium Standard Solution, 50-mg/L as Cr^{3+}, to 1000 mL with deionized water. Prepare this solution daily.
2. To adjust the calibration curve using the reading obtained with the 0.25-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.

3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision

Standard: 0.25 mg/L Cr

Program	95% Confidence Limits of Distribution
100	0.24–0.26 mg/L Cr

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.01 mg/L Cr

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Trivalent chromium in the sample is oxidized to the hexavalent form by hypobromite ion under alkaline conditions. The sample is acidified. The total chromium content is determined by the 1,5-Diphenylcarbohydrazide method. Determine trivalent chromium by subtracting the results of a separate hexavalent chromium test from the results of the total chromium test. Test results are measured at 540 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Total Chromium Reagent Set (100 tests)			22425-00
Includes:			
Acid Reagent Powder Pillows	1 pillow	100/pkg	2126-99
ChromaVer® 3 Chromium Reagent Powder Pillows	1 pillow	100/pkg	12066-99
Chromium 1 Reagent Powder Pillows	1 pillow	100/pkg	2043-99
Chromium 2 Reagent Powder Pillows	1 pillow	100/pkg	2044-99

Required Apparatus

Hot plate, 3½-inch diameter, 120 VAC, 50/60 Hz		each	12067-01
or			
Hot plate, 4 inch diameter, 240 VAC, 50/60 Hz		each	12067-02
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Water bath and rack	1	each	1955-55

Required Standards

Chromium, Trivalent, Standard Sol., 50-mg/L Cr^{3+}		100 mL	14151-42
Chromium, Trivalent, Standard Sol., 50-mg/L Cr^{3+}, Voluette Ampule®, 10-mL		16/pkg	14257-10

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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Chromium, Total

1,5-Diphenylcarbohydrazide Method

UniCell™ Vials

(0.03 to 1.00 mg/L Tot-Cr)

Scope and Application: For water and wastewater

Tips and Techniques

- Adjust pH of preserved samples to between pH 3–9 before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check*.
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is between 15–25 °C.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

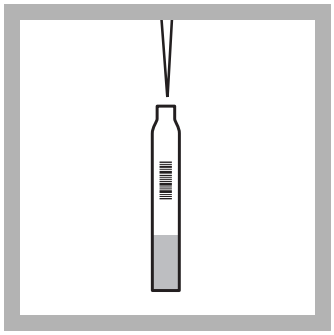
1. Touch

Hach Programs.

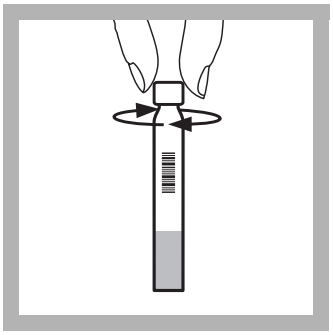
Select program

803 Chromium, HCT 156

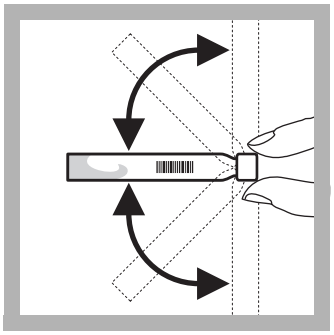
Touch **Start**.



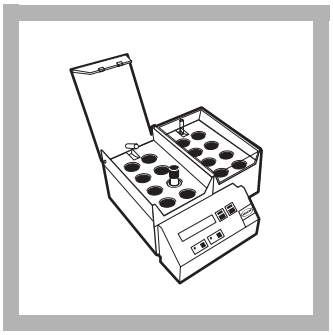
2. Pipet 4.0 mL of sample into a sample vial.



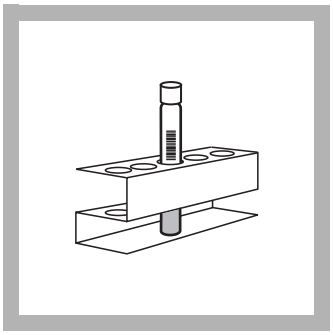
3. Screw a **blue** UniCap A (HCT 156 A) onto the sample vial.



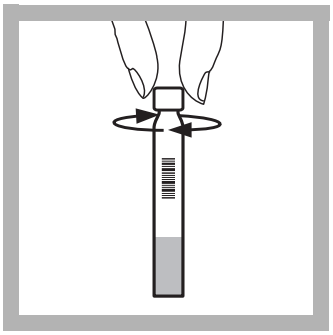
4. Invert the sample vial several times to mix.



5. Heat the vial in the reactor block for 60 minutes at 100 °C.

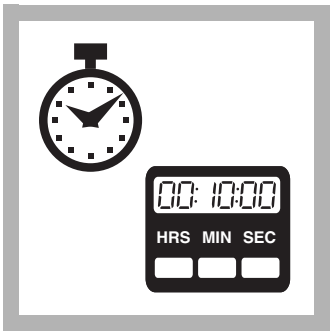


6. After the heating period, remove the vial from the reactor block and place it in a cooling rack.



7. When the vials have cooled to room temperature, screw an **orange** UniCap B (HCT 156 B) onto the sample vial.

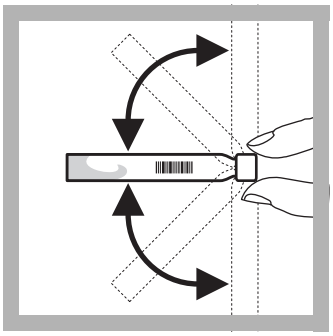
Invert the sample several times to mix.



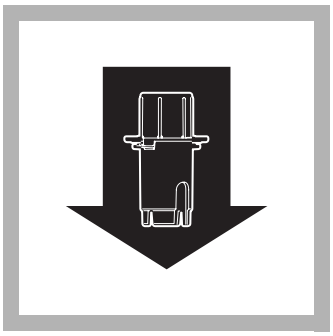
8. Touch the timer icon.

Touch **OK**.

A 10-minute reaction period will begin.



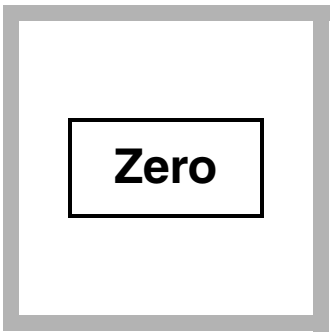
9. After the reaction period, invert the sample vial to mix.



10. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

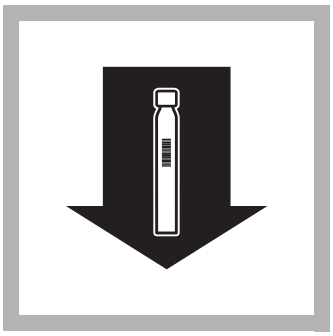
Place the zero vial into the cell adapter.



11. Touch **Zero**.

The display will show:

0.00 mg/L Tot–Cr
Underrange



12. Place the sample vials into the celladapter.

Touch **Read**.

Results will appear in mg/L Tot-Cr.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Cl^-	1000 mg/L
Ca^{2+}	125 mg/L
Mg^{2+}, NH_4^+	100 mg/L
Zn^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}	50 mg/L

(continued)

Interfering Substance	Interference Levels
Ag^{+}, Pb^{2+}	25 mg/L
Cu^{2+}, Fe^{3+}	10 mg/L
Sn^{2+}	1 mg/L

Larger amounts of iron, copper, reducing or oxidizing agents give low results. Lead, mercury, and tin give high results.

Note: *Undissolved chromium is not determined with the determination of chromium VI. Total Chromium can only be determined after digestion.*

Note: *Concentrations above 20 mg/L produce result displays within the ranges given above. In such cases, verify the results by dilution.*

Sample Collection, Preservation, and Storage

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately or if the samples contain only chromium(VI) ions. To preserve the sample for Total Chromium, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3 and 9 with 5.0 N sodium hydroxide standard solution.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample vial (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Pipet 0.1 mL, 0.2 mL, and 0.3 mL of 50-mg/L Cr (VI) standard, respectively, to three 25-mL samples and mix thoroughly.
5. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 0.70-mg/L Cr^{6+} standard solution daily, as follows:

1. Using a pipet to transfer 1.40 mL of Hexavalent Chromium Standard Solution, 50-mg/L, into a Class A 100-mL volumetric flask.
2. Dilute to the mark with deionized water.

3. To adjust the calibration curve using the reading obtained with the 0.70-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Perform the hexavalent chromium procedure as described above. See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.50 mg/L Cr^{6+}

Program	95% Confidence Limits of Distribution
803	0.38–0.62 mg/L Cr^{6+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
803	Entire range	0.010	0.01 mg/L Cr^{6+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chromium(VI) ions react with 1,5-diphenylcarbazide to yield 1,5-di-phenylcarbazone and Chromium(III), which forms a red complex. Measurements are taken at 543 nm.

Required Reagents

Description	Unit	Cat. No.
Chromium - Cr (VI), UniCell™ HCT 156	23/pkg	HCT 156

Optional Reagents

Chromium Standard, 50-mg/L	100 mL	810-42 H

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Digital Reactor Block	each	DRB 100
Flask, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 100-mL	each	20886-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	400/pkg	27950-00
pH Paper	100/pkg	26013-00
Test tube rack, cooling	each	18641-00

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Method 8078 **1-(2-Pyridylazo)-2-Naphthol (PAN) Method***

Powder Pillows **(0.01 to 2.00 mg/L)**

Scope and Application: For water and wastewater; digestion is required for determining total recoverable cobalt (see *Section 4*); if EDTA is present, use the vigorous digestion.

* Adapted from Watanbe, H., *Talanta*, 21 295 (1974)

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- If the sample is less than 10 °C (50 °F), warm to room temperature prior to analysis.
- You can determine nickel with the same sample prepared with this method. Use Program Number 340. A reagent blank is necessary for the nickel procedure.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows Method 8078

1. Touch

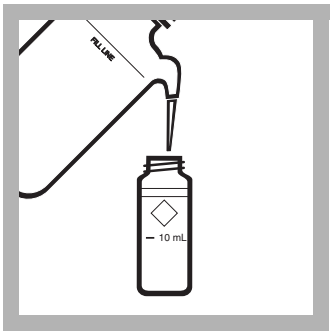
Hach Programs.

Select program

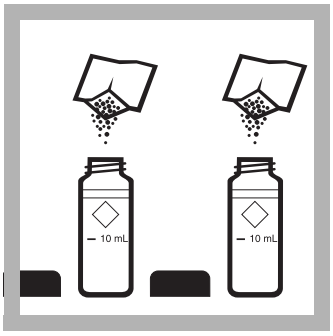
110 Cobalt.

Touch **Start**.

2. Fill a round sample cell to the 10-mL mark with sample. (This is the prepared sample.)

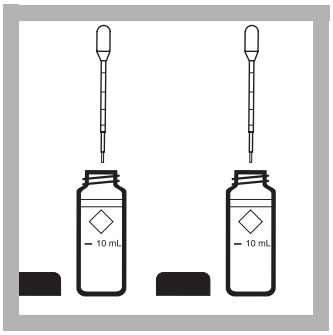


3. Fill another round sample cell to the 10-mL mark with deionized water. (This is the blank.)

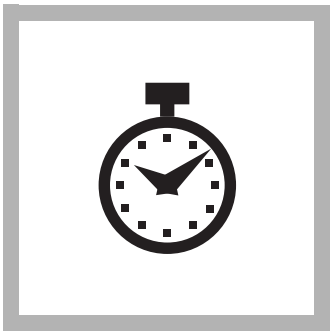


4. Add the contents of one Phthalate-Phosphate Reagent Powder Pillow to each cell. Cap. Immediately shake to dissolve.

If the sample contains iron (Fe^{+3}), make sure that all of the powder is completely dissolved before continuing with *step 5*.



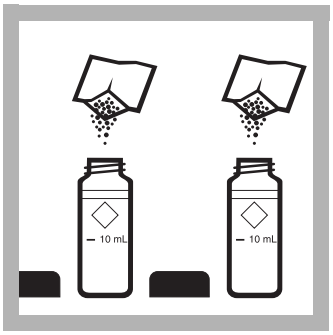
5. Use the plastic dropper provided to add 0.5 mL of 0.3% PAN Indicator Solution to each cell. Stopper each cell. Invert several times to mix.



6. Touch the timer icon.

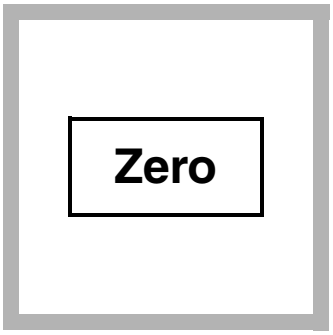
Touch **OK**.

A three-minute reaction period will begin. During the reaction period, the sample solution may vary from green to dark red, depending on the chemical make-up of the sample. The deionized water blank should be yellow.



7. When the timer beeps, add the contents of one EDTA Reagent Powder Pillow to each cylinder. Stopper. Shake to dissolve.

8. Place the blank into the cell holder.

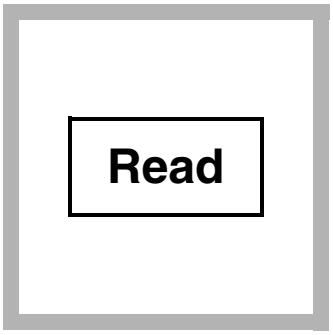


9. Touch **Zero**.

The display will show:

0.00 mg/L Co

10. Place the prepared sample into the cell holder.



11. Touch **Read**.

Results will appear in mg/L Co.

Interferences

Interfering Substance	Interference Levels and Treatments
Al^{3+}	32 mg/L
Ca^{2+}	1000 mg/L as $CaCO_3$
Cd^{2+}	20 mg/L
Cl^{-}	8000 mg/L
Cr^{3+}	20 mg/L
Cr^{6+}	40 mg/L
Cu^{2+}	15 mg/L
F^{-}	20 mg/L
Fe^{2+}	Interferes directly and must not be present
Fe^{3+}	10 mg/L
K^{+}	500 mg/L
Mg^{2+}	400 mg/L
Mn^{2+}	25 mg/L
Mo^{6+}	60 mg/L
Na^{+}	5000 mg/L
Pb^{2+}	20 mg/L
Zn^{2+}	30 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see *Section 3.3 Interferences*.

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (Cat. No. 2540-49) (about 5 mL per liter). Preserved samples can be stored up to six months at room temperature. Adjust the sample pH between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-32) just before analysis. Do not exceed pH 8 as this may cause some loss of cobalt as a precipitate. Correct test results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29* for more information.

Accuracy Check

Standard Solution Method

Prepare a 1.0 mg/L cobalt standard solution as follows:

1. Dilute 10.0 mL of a 10-mg/L working stock solution to 100 mL in a volumetric flask. Prepare the 10-mg/L working stock solution daily by diluting 10.00 mL of Cobalt Standard Solution, 1000-mg/L as Co, to 1000 mL with deionized water.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 1.00 mg/L Co

Program	95% Confidence Limits of Distribution
110	0.98–1.02 mg/L Co

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.01 mg/L Co

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

After buffering the sample and masking any Fe^{3+} with pyrophosphate, the cobalt is reacted with 1-(2-Pyridylazo)-2-Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal-PAN complexes except nickel and cobalt, which can both be determined using the same sample. Test results are measured at 620 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Cobalt Reagent Set, 10-mL (100 tests)			26516-00
Includes:			
(2) EDTA Reagent Powder Pillows	2 pillows	100/pkg	7005-99
(2) Phthalate-Phosphate Reagent Powder Pillows	2 pillows	100/pkg	26151-99
(1) PAN Indicator Solution, 0.3%	1 mL	100 mL	21502-32
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Cylinder, graduated mixing, 25-mL	2	each	20886-40
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Cobalt Standard Solution, 1000-mg/L Co		100 mL	21503-42

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DR/2400 Color, True and Apparent

Method 8025 **Platinum-Cobalt Standard Method* ****

(5 to 500 units)

Scope and Application: For water, wastewater, and seawater; equivalent to NCASI method 253 for pulp and paper effluent using 465 nm (requires pH adjustment)

* Adapted from *Standard Methods for the Examination of Water and Wastewater* and *NCASI, Technical Bulletin No. 253*, Dec. 1971
** Adapted from *Wat. Res.* Vol. 30, No. 11, pp. 2771–2775, 1996

Tips and Techniques

- NCASI procedure requires pH adjustment. Adjust the pH to 7.6 with 1.0 N HCl or 1.0 N NaOH. When adjusting the pH, if overall volume change is greater than 1%, start over and use a stronger acid or base.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- To test for **apparent color**, omit steps 3, 4, 5, and 7. Do not filter the sample. Use unfiltered deionized water for the blank.

Method 8025

1. Touch

Hach Programs.

Select program

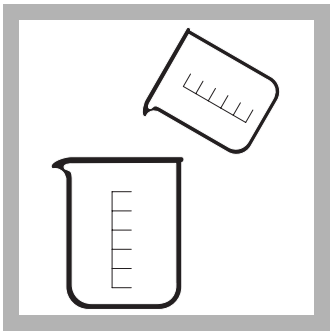
120 Color, 455 nm

or

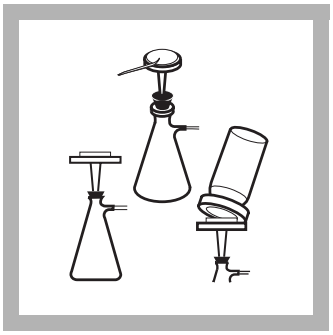
125 Color, 465 nm.

Touch **Start**.

Note: *Use Hach program 125 when performing the NCASI procedure.*

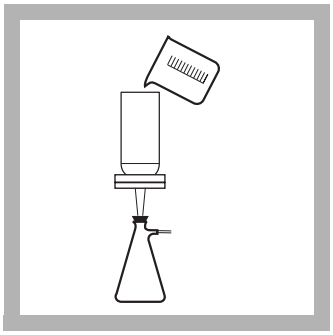


2. Collect 200 mL of sample in a 400-mL beaker. For the NCASI procedure, adjust the pH as described above.

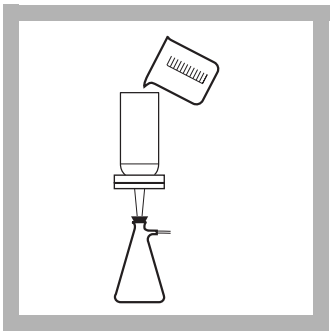


3. Assemble the filtering apparatus (0.45 micron membrane filter, filter holder, filter flask, and aspirator).

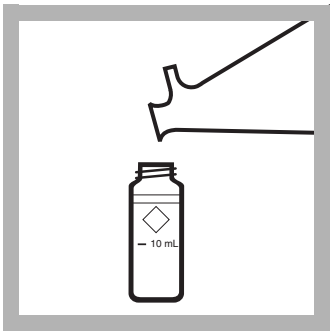
Note: *NCASI prescribes a 0.8-micron filter.*



4. Rinse the filter by pouring about 50 mL of deionized water through the filter. Discard the rinse water.

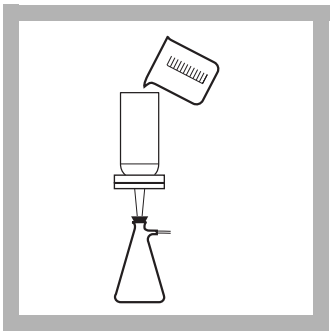


5. Pour another 50 mL of deionized water through the filter.

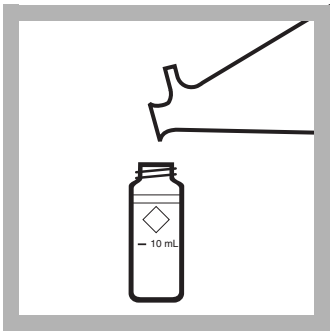


6. Fill a round sample cell with 10 mL of filtered deionized water from s*tep 5*. (This is the blank.)

Discard the excess water in the flask.

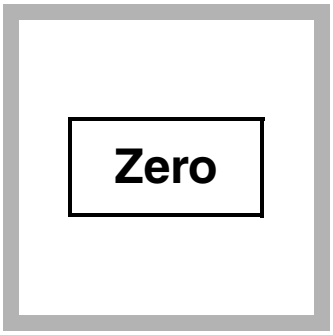


7. Pour about 50 mL of sample through the filter.



8. Fill a second round sample cell with 10 mL of filtered sample. (This is the prepared sample.)

9. Wipe the blank and place it into the cell holder.

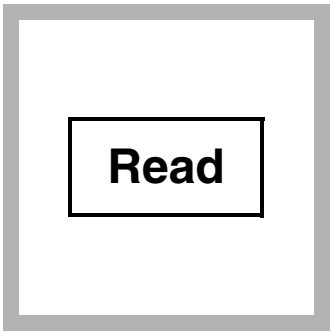


10. Touch **Zero**.

The display will show:

0 units PtCo

11. Wipe the prepared sample and place it into the cell holder.



12. Touch **Read**.

Results will appear in mg/L PtCo.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, fill bottles completely and cap tightly. Avoid excessive agitation or prolonged contact with air. Samples can be stored for 24 hours by cooling to 4 °C (39 °F). Warm to room temperature before analysis.

Accuracy Check

Standard Solution Method

Prepare a 250 platinum-cobalt units standard as follows:

1. Using Class A glassware, pipet 50.00 mL of a 500 Platinum-Cobalt Units Standard Solution into a 100-mL volumetric flask. Dilute to the 100 mL mark with deionized water.
2. To adjust the calibration curve using the reading obtained with the 250 platinum-cobalt units standard, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected units). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 250 units Pt-Co

Program	95% Confidence Limits of Distribution
120	245–255 units Pt-Co
125	245–255 units Pt-Co

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
120	Entire Range	0.010	17 units Pt-Co
125	Entire Range	0.010	17 units Pt-Co

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Color may be expressed as “apparent” or “true” color. The apparent color includes that from dissolved materials plus that from suspended matter. By filtering or centrifuging out the suspended materials, the true color can be determined. The procedure describes true color analysis. If apparent color is desired, it can be determined by measuring an unfiltered water sample. The stored program is used for both forms of color.

The stored program is calibrated in color units based on the APHA-recommended standard of 1 color unit being equal to 1 mg/L platinum as chloroplatinate ion. Test results for Programs 120 and 125 are measured at 455 and 465 nm, respectively.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Hydrochloric Acid Solution, 1.0 N		1 liter	23213-53
Sodium Hydroxide, 1.00 N		900 mL	1045-53
Water, deionized	50 mL	4 liters	272-56

Required Apparatus

Aspirator, Nalgene vacuum pump	1	each	2131-00
Filter Holder, 47-mm, 300-mL graduated	1	each	13529-00
Filter, membrane, 47-mm, 0.8-microns	1	100/pkg	26408-00
Filter, membrane, 47-mm, 0.45-microns	1	100/pkg	13530-00
Flask, filtering, 500-mL	1	each	546-49
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Stopper, rubber, one hole, No. 7	1	6/pkg	2119-07
Tubing, rubber	1	12 ft	560-19

Required Standards

Color Standard Solution, 500 platinum-cobalt units		1 liter	1414-53
Color Standard Solution, 15 platinum-cobalt units		1 liter	26028-53
Color Standard Solution, 500 platinum-cobalt units, 10-mL Voluette® Ampules		16/pkg	1414-10

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

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★Method 8506 and Method 8026

Bicinchoninate Method*

Powder Pillows or AccuVac® Ampuls

(0.04 to 5.00 mg/L)

Scope and Application: For water, wastewater and seawater**; Method 8506 USEPA approved for reporting wastewater analysis (digestion required; See *Section 4*)***

* Adapted from Nakano, S., *Yakugaku Zasshi*, 82 486-491 (1962) [*Chemical Abstracts*, 58 3390e (1963)]
** Pretreatment required; see *Interferences (Using Powder Pillows)*
*** *Federal Register*, 45 (105) 36166 (May 29, 1980)

Tips and Techniques

- Digestion is required for determining total copper. See *Section 4* for the digestion procedure.
- The chemistry is pH sensitive. Adjust the pH of acid-preserved samples to 4–6 with 8 N KOH before analysis.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- If copper is present, the sample will turn purple when it mixes with the reagent powder.
- Accuracy is not affected by undissolved powder.

Powder Pillows — Method 8506

1. Touch

Hach Programs.

Select program

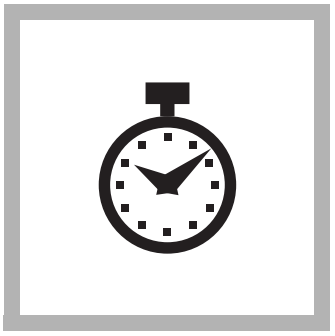
135 Copper, Bicin.

Touch **Start**.

2. Fill a sample cell with 10 mL of sample.

3. Add the contents of one CuVer® 1 Copper Reagent Powder Pillow to the sample cell (the prepared sample). Cap and invert to mix.

Note: *Use a CuVer 2 Copper Reagent Pillow for samples containing high levels of aluminum, iron, and hardness. A 25-mL sample cell is required. See Table 1.*

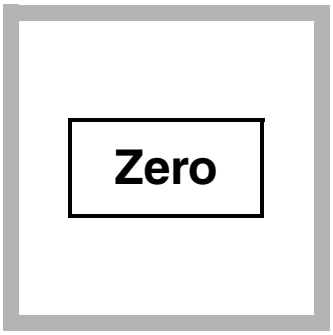


4. Touch the timer icon.

Touch **OK**.

A 2-minute reaction period will begin.

5. When the timer beeps, fill a second sample cell (the blank) with 10 mL of sample. Place the blank into the cell holder.

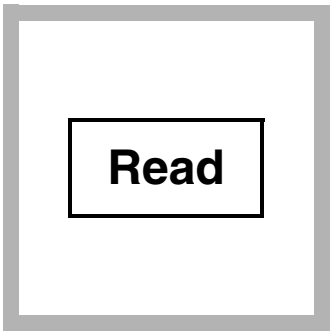


6. Touch **Zero**.

The display will show:

0.00 mg/L Cu

7. Within 30 minutes after the timer beeps, place the prepared sample into the cell holder.



8. Touch Read.

Results will appear in mg/L Cu.

AccuVac Ampul — Method 8026

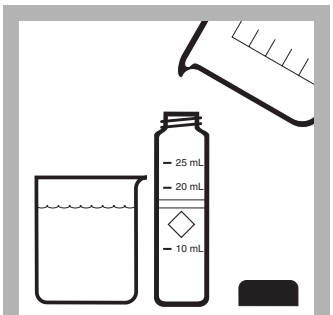
1. Touch

Hach Programs.

Select program

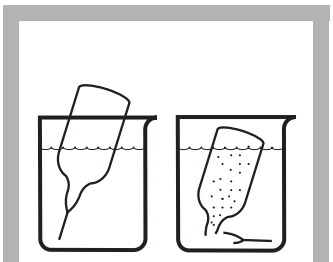
140 Copper, Bicin. AV.

Touch **Start**.

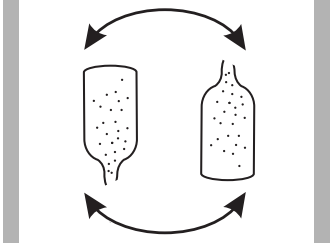


2. Collect at least 40 mL of sample in a 50-mL beaker.

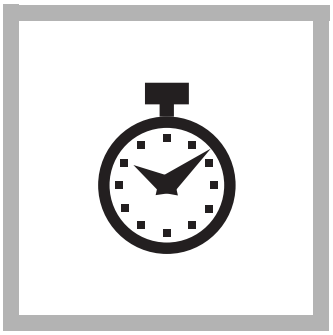
Fill a round sample cell with 25-mL of sample (the blank).



3. Fill a CuVer 2 AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampule several times to mix. Wipe off any liquid or fingerprints with cloth or soft paper towel.

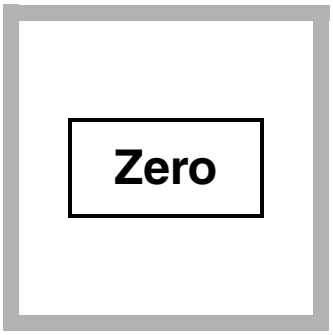


5. Touch the timer icon.

Touch **OK**

A 2-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder. Close the cover.



7. Touch **Zero**.

The display will show:

0.00 mg/L Cu

8. Within 30 minutes after the timer beeps, place the AccuVac Ampul into the cell holder.

Touch **Read**.

Results will appear in mg/L Cu.

Interferences

To differentiate free copper from that complexed to EDTA or other complexing agents, use a Free Copper Reagent Powder Pillow in place of the CuVer 1 Powder Pillow in step 3. Results in *step 8* will be free copper only. Add a Hydrosulfite Reagent Powder Pillow to the same sample and re-read the result. This result will include the total dissolved copper (free and complexed). Unlike CuVer 1 Reagent, CuVer 2 Reagent Powder Pillows and AccuVac Ampuls react directly with copper, which is complexed by chelants such as EDTA.

Table 1 Interfering Substances and Suggested Treatments for Powder Pillows

Interfering Substance	Interference Levels and Treatments
Acidity	If the sample is extremely acidic (pH 2 or less) a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution (Cat. No. 282-32H) drop-wise while swirling to dissolve the turbidity. Continue with step 2.
Aluminum, Al^{3+}	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow (Cat. No. 21882-99) for the CuVer 1 Pillow used in step 3. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample cell.
Cyanide, CN^-	Prevents full color development. Before adding the CuVer 1 Powder Pillow Reagent, add 0.2 mL of formaldehyde (Cat. No. 2059-32) to the 10-mL sample. Wait 4 minutes before taking the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow (Cat. No. 21882-99) for the CuVer 1 Pillow used in step 3. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample cell.
Iron, Fe^{3+}	Follow the powder pillow procedure above, but substitute a CuVer 2 Copper Reagent Powder Pillow (Cat. No. 21882-99) for the CuVer 1 Pillow used in step 3. Results obtained will include total dissolved copper (free and complexed). Requires a 25-mL sample cell.
Silver, Ag^+	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution (Cat. No. 765-42) to 75 mL of sample, followed by filtering through a fine or highly retentive filter. Use the filtered sample in the procedure.

Table 2 Interfering Substances and Suggested Treatments for AccuVac® Ampuls

Interfering Substance	Interference Levels and Treatments
Acidity	If the sample is extremely acidic (pH 2 or less) a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution (Cat. No. 282-32) drop-wise until sample pH is above 4. Continue with step 2.
Aluminum, Al^{3+}	Reagents accommodate high levels.
Cyanide, CN^-	Prevents full color development. Add 0.5 mL of formaldehyde (Cat. No. 2059-32) to a 25-mL sample before using CuVer 2 Reagent AccuVac Ampul. Wait 4 minutes before taking the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Reagents accommodate high levels.
Iron, Fe^{3+}	Reagents accommodate high levels.
Silver, Ag^+	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution (Cat. No. 765-42) to 75 mL of sample, followed by filtering through a fine or highly retentive filter. Use the filtered sample in the procedure.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. Adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter) (Cat. No. 2540-49). Store preserved samples up to six months at room temperature. Before analysis, adjust the pH to 4–6 with 8 N Potassium Hydroxide (Cat. No. 282-32). Do not exceed pH 6, as copper may precipitate. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*. If only dissolved copper is to be determined, filter the sample before acid addition.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off of a Copper Voluette® Ampule Standard, 12.5-mg/L Cu.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Touch the timer icon. After the timer beeps, read the result.
6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Touch the timer icon. After the timer beeps, read the result.
7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Touch the timer icon. After the timer beeps, read the result. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac Ampuls, fill three mixing cylinders (Cat. No. 1896-41) with 50-mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of Copper Voluette Ampule Standard, 75-mg/L Cu. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure*

above. Accept each standard additions reading by touching **Read**. *Each addition should reflect approximately 100% recovery.*

8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

Prepare a 4.00-mg/L Standard as follows:

1. Using Class A glassware, pipet 4.00 mL of Copper Standard Solution, 100-mg/L as Cu, into a 100-mL volumetric flask. Dilute to volume with deionized water, stopper and invert to mix.
2. To adjust the calibration curve using the reading obtained with the 4.00-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 1.000 mg/L Cu

Program	95% Confidence Limits of Distribution
135	0.96–1.04 mg/L Cu
140	0.96–1.04mg/L Cu

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
135	Entire range	0.010	0.04 mg/L Cu
140	Entire range	0.010	0.04 mg/L Cu

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Copper in the sample reacts with a salt of bicinchoninic acid contained in CuVer 1 or CuVer 2 Copper Reagent to form a purple colored complex in proportion to the copper concentration. Test results are measured at 560 nm.

Copper

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
CuVer® 2 Copper Reagent AccuVac® Ampuls	1 ampul	25/pkg	25040-25
or			
CuVer® 1 Copper Reagent Powder Pillows	1 pillow	100/pkg	21058-69

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Copper Standard Solution, 100-mg/L as Cu	100 mL	128-42
Copper Voluette® Ampule Standard, 12.5-mg/L as Cu	16/pkg	21126-10
Copper Voluette® Ampule Standard, 75-mg/L as Cu, 2-mL	10/pkg	14247-10

Optional Standards

Reagent Set for Free and Total Copper		24392-00
Includes:		
Hydrosulfite Reagent Powder Pillows		21188-69
Free Copper Reagent Powder Pillows		21823-69
Sample Cells, 10-20-25 mL, w/cap	6/pkg	24019-06
CuVer® 2 Copper Reagent Powder Pillows, 25-mL	100/pkg	21882-99

Method 8143 **Porphyrin Method***

Powder Pillows **LR (2 to 210 µg/L)**

Scope and Application: For water, wastewater, and sea water

* Adapted from Ishii and Koh, *Bunseki Kagaku*, 28 (473), 1979

Tips and Techniques

- Digestion is required for determining total copper. See *Section 4* for digestion procedure.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wash all glassware with detergent. Rinse with tap water. Rinse again with 1:1 Nitric Acid Solution. Rinse a third time with copper-free, deionized water.
- If samples contain high levels of metals, a slight metallic deposit or yellow buildup may form in the sample cell. Wash the cell as described above.

Powder Pillows Method 8143

1. Touch

Hach Programs.

Select program

145 Copper, Porphyrin.

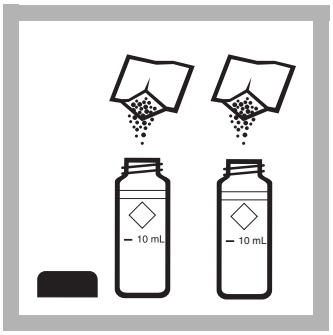
Touch **Start**.

2. Fill two round sample cells with 10 mL of sample.

3. Add the contents of one Copper Masking Reagent Powder Pillow to one of the sample cells (the blank). Swirl to dissolve.

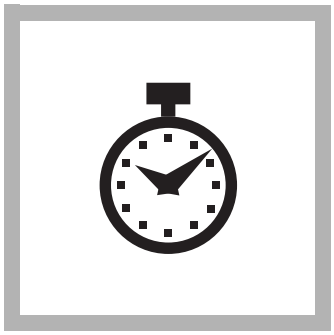
The second sample cell is the prepared sample.

4. Add the contents of one Porphyrin 1 Reagent Powder Pillow to each sample cell. Swirl to dissolve.



5. Add the contents of one Porphyrin 2 Reagent Powder Pillow to each sample cell. Swirl to dissolve.

Note: *If copper is present, the sample will momentarily turn blue, then return to yellow.*

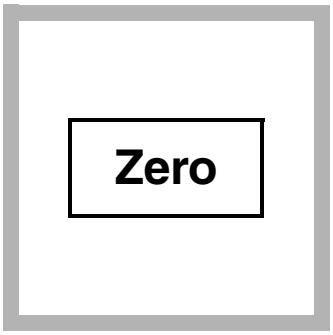


6. Touch the timer icon.

Touch **OK**.

A 3-minute reaction period will begin.

7. When the timer beeps, place the blank into the cell holder.

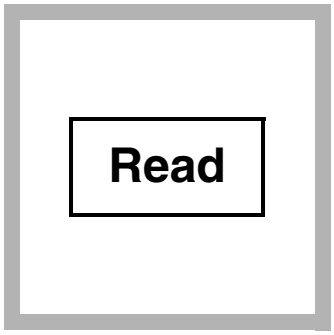


8. Touch **Zero**.

The display will show:

0 µg/L Cu

9. Place the prepared sample into the cell holder.



10. Touch **Read**.

Results will appear in µg/L Cu.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum, Al^{3+}	60 mg/L
Cadmium, Cd^{2+}	10 mg/L
Calcium, Ca^{2+}	1500 mg/L
Chelating agents	Interfere at all levels unless either the Digesdahl or vigorous digestion is performed (see *Section 4* on page *55*).
Chloride, Cl^-	90,000 mg/L
Chromium, Cr^{6+}	110 mg/L
Cobalt, Co^{2+}	100 mg/L
Fluoride, F^-	30,000 mg/L
Iron, Fe^{2+}	6 mg/L
Lead, Pb^{2+}	3 mg/L
Magnesium	10,000 mg/L
Manganese	140 mg/L
Mercury, Hg^{2+}	3 mg/L
Molybdenum	11 mg/L
Nickel, Ni^{2+}	60 mg/L
Potassium, K^+	60,000 mg/L
Sodium, Na^+	90,000 mg/L
Zinc, Zn^{2+}	9 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; (see *Section 3.3 Interferences* on page *41*.)

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. To preserve, adjust the pH to 2 or less with nitric acid (about 5 mL per liter). Store preserved samples up to six months at room temperature. Before testing, adjust the pH of the preserved sample to between 2 and 6. If the sample is too acidic, adjust the pH with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-32). Correct test results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Prepare a 4000-µg/L copper standard by adding 4.00 mL Copper Standard Solution, 100.0-mg/L, to a 100-mL volumetric flask. Dilute to 100 mL with copper-free deionized water.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.

4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Fill eight sample cells with 10 mL of sample. Use the TenSette® Pipet to add 0.1 mL of Copper Standard Solution, 4000-µg/L Cu, to two of the sample cells. Then pipet 0.2 mL of the standard solution into two more cells. Finally, pipet 0.3 mL of the standard solution into two more cells.
6. Analyze each standard addition sample as described in the procedure above, using one of the two spiked samples in each set as the blank. Accept each standard additions reading by touching **Read**. The copper concentration reading should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

1. To assure the accuracy of the test, prepare a 150-µg/L copper standard by pipetting 15.00 mL of Copper Standard Solution, 10.0-mg/L Cu, into a 1000-mL volumetric flask.
2. Dilute to the mark with copper-free, reagent-grade water. Prepare this solution daily. Perform the copper procedure as described above.
3. To adjust the calibration curve using the reading obtained with the 150-µg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 50 µg/L Cu

Program	95% Confidence Limits of Distribution
145	45–55 µg/L Cu

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	2 µg/L Cu

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The porphyrin method is very sensitive to trace amounts of free copper. The method is free from most interferences and does not require any sample extraction or concentration before analysis. Interferences from other metals are eliminated by the copper masking reagent. The porphyrin indicator forms an intense, yellow-colored complex with any free copper present in sample. Test results are measured at 425 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Copper Reagent Set (100 Tests)			26033-00
Includes:			
(1) Copper Masking Reagent Powder Pillows	1 pillow	100/pkg	26034-49
(2) Porphyrin 1 Reagent Powder Pillows	2 pillows	100/pkg	26035-49
(2) Porphyrin 2 Reagent Powder Pillows	2 pillows	100/pkg	26036-49
Nitric Acid Solution, 1:1		500 mL	2540-49

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Copper Standard Solution, 100-mg/L Cu		100 mL	128-42
Water, deionized		4 liters	272-56
Copper, Standard Solution, 10-mg/L Cu		100 mL	129-32

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3/04 1ed

Copper

Bathocuproine Method

UniCell™ Vials (0.10 to 6.00 mg/L)

Scope and Application: For water, wastewater, raw water, and process control

Tips and Techniques

- Undissolved copper and copper contained in complexes can only be determined after digestion with the Metal Prep Set (HCT 200). The measuring range for total copper is 0.12–7.20 mg/L.
- Adjust pH of preserved samples to between pH 3–9 before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check*.
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is between 15–25 °C.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

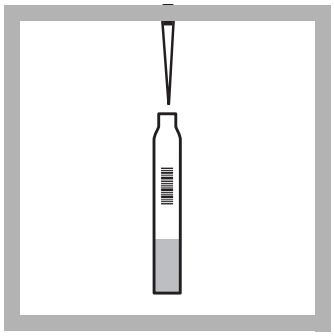
1. Touch

Hach Programs.

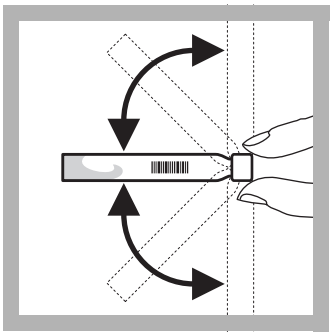
Select program

146 Copper, HCT 163

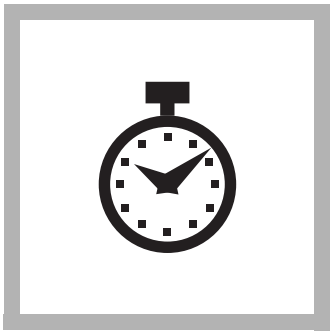
Touch **Start**.



2. Pipet 4 mL of sample into a sample vial.



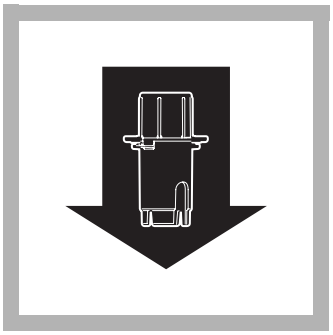
3. Cap and invert the sample vial until the solid in the vial dissolves completely.



4. Touch the timer icon.

Touch **OK**.

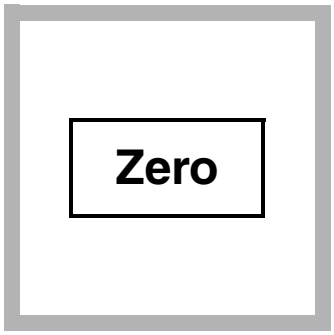
A 3-minute reaction period will begin.



5. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

When the timer beeps, wipe the zero vial and place it into the cell holder.



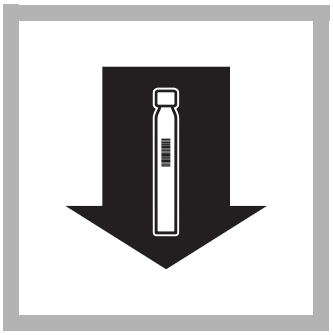
6. Touch **Zero**.

The display will show:

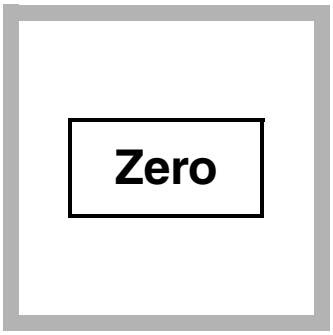
0.00 mg/L Free Cu

Underrange

Note: *If the sample was pretreated with the* Metal Prep Set (HCT 200)*, touch* **Options** *and select* **Tot. Cu**.



7. Wipe the sample vial and place it into the cell holder.



8. Touch **Read**.

Results will appear in mg/L Cu.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Cl^-, NO_3^-, SO_4^{2-}	1000 mg/L
Mg^{2+}, NH^{4+}, Ca^{2+}, PO_4^{3-}, CO_3^{2-}, NO^{2-}, K^+, Na^+	500 mg/L
Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}	50 mg/L
Cr^{3+}, Cr^{6+}	25 mg/L
Fe^{2+}, Fe^{3+}	15 mg/L
Sn^{2+}, Hg^{2+}	5 mg/L

Note: *Higher amounts of iron and chromium cause a positive interference.*

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned or plastic containers. Acid addition is not necessary if samples are analyzed immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3 and 6 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 6 or copper may precipitate.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three sample spikes. Fill three mixing cylinders with 100 mL of sample. Pipet 0.1 mL, 0.2 mL, and 0.3 mL of 1000-mg/L Cu standard, respectively, to each sample and mix each thoroughly.
5. Transfer 4 mL of each solution to three sample vials and analyze as described in the procedure. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

Prepare a 2.00-mg/L Standard as follows:

1. Pipet 0.2 mL of 1000-mg/L Cu into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the copper procedure as described.
2. To adjust the calibration curve using the reading obtained with the 2.00-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 2.00 mg/L Free Cu

Program	95% Confidence Limits of Distribution
146	1.76–2.24 mg/L Free Cu

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
146	Entire range	0.010	0.04 mg/L Free Cu

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Copper (I) ions form an orange complex with the disodium salt of bathocuproine disulphonic acid. Any copper (II) ions present in the water sample are reduced to copper (I) ions by ascorbic acid before the complex is formed. Measurements are taken at 478 nm.

Required Reagents

Description	Unit	Cat. No.
Copper - Cu, UniCell™ HCT 163	23/pkg	HCT 163

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Graduated cylinder, mixing, 100-mL	each	20886-42
Flask, volumetric, 100-mL	each	14574-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pk/100	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	pk/400	27950-00
pH Paper	pk/100	26013-00

Optional Standards

Metal Prep Set, HCT 200	50 digestions	HCT 200
Copper Standard Solution, 1000-mg/L as Cu	100 mL	2593-42
Sodium Hydroxide, 5 N	1 liter	2450-53
Nitric Acid Solution, ACS Grade	500 mL	152-49

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Method 8027

Pyridine-Pyrazalone Method*

Powder Pillows

(0.001 to 0.240 mg/L CN^-)

Scope and Application: For water, wastewater, and seawater

* Adapted from Epstein, Joseph, *Anal. Chem.* 19(4), 272 (1947)

Tips and Techniques

- Use a water bath to maintain the optimum temperature for the reaction in this test (25 °C). Samples at less than 23 °C require longer reaction times, and samples at greater than 25 °C yield low results.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- The timing for *steps 2* through *8* is critical. You may find it useful to open the necessary reagents before starting this sequence.
- All samples to be analyzed for cyanide should be treated by acid distillation except when experience has shown that there is no difference in results obtained with or without distillation. See *Acid Distillation on page 5*.
- See *Pollution Prevention and Waste Management on page 3* for proper disposal of solutions containing cyanide.

Powder Pillows Method 8027

1. Touch

Hach Programs.

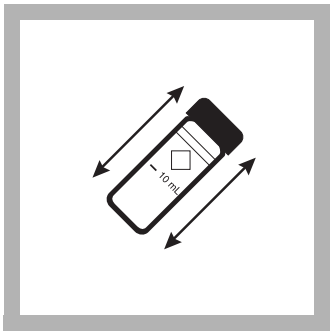
Select program

160 Cyanide.

Touch **Start**.

2. Using a graduated cylinder, fill a round sample cell with a 10 mL of sample.

3. Add the contents of one CyaniVer 3 Cyanide Reagent Powder Pillow. Cap. (This is the prepared sample.)

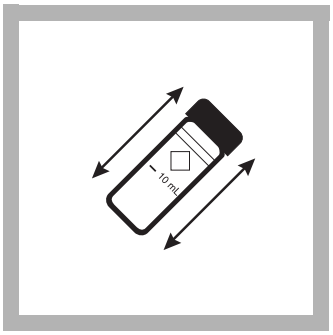


4. Shake the sample cell for 30 seconds.

5. Leave the sample cell undisturbed for an additional 30 seconds.

6. Add the contents of one CyaniVer 4 Cyanide Reagent Powder Pillow.

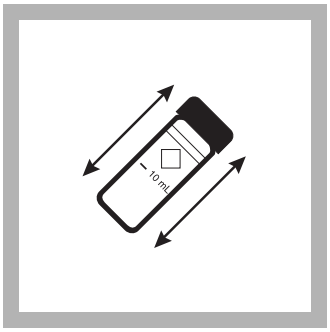
Cap the sample cell.



7. Shake the sample for 10 seconds. Immediately proceed to *step 8*. (Delaying the addition of the CyaniVer 5 will produce low test results.)

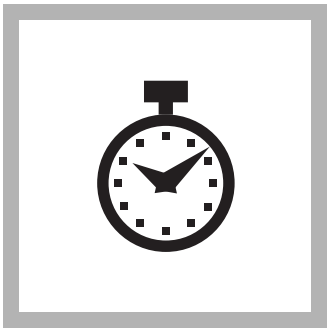
8. Add the contents of one CyaniVer 5 Cyanide Reagent Powder Pillow.

Cap the sample cell.



9. Shake the cell vigorously.

If cyanide is present, a pink color will develop.



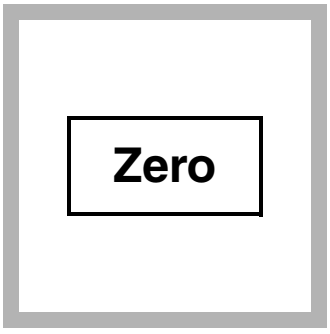
10. Touch the timer icon. Touch **OK**.

A 30-minute reaction period will begin.

The solution will turn from pink to blue.

11. When the timer beeps, fill another round sample cell with 10 mL of sample. (This is the blank.)

12. Wipe the blank and place it into the cell holder.

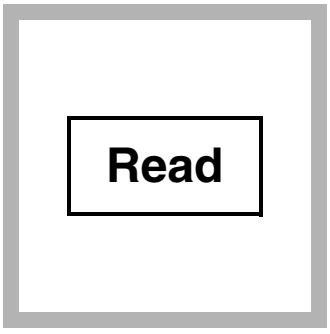


13. Touch **Zero**.

The display will show:

0.000 mg/L CN^-

14. Wipe the prepared sample and place it into the cell holder.



15. Touch **Read**.

Results will appear in mg/L CN^-.

Pollution Prevention and Waste Management

Special Considerations for Cyanide Containing Materials

Samples analyzed by this procedure may contain cyanide, which is regulated as reactive (D003) waste by the federal RCRA. It is imperative these materials be handled safely to prevent the release of hydrogen cyanide gas (an extremely toxic material with the smell of almonds). Most cyanide compounds are stable and can be safely stored for disposal in highly alkaline solutions (pH >11) such as 2 N sodium hydroxide. Never mix these wastes with other laboratory wastes which may contain lower pH materials such as acids or even water.

In the event of a spill or release, special precautions must be taken to prevent exposure to hydrogen cyanide gas. The following steps may be taken to destroy the cyanide compounds in the event of an emergency:

- Use a fume hood or supplied air or self contained breathing apparatus.
- While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and calcium hypochlorite or sodium hypochlorite (household bleach).
- Maintain a strong excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.
- Neutralize and flush the solution down the drain with a large excess of water. Note: if the solution contains other regulated materials such as chloroform or heavy metals, it may still need to be collected for hazardous waste disposal. Never flush hazardous wastes down the drain.

Interferences

Interfering Substance	Interference Levels and Treatments
Chlorine	Large amounts of chlorine in the sample will cause a milky white precipitate after the addition of the CyaniVer® 5 Reagent. If chlorine or other oxidizing agents are known to be present, pretreat the sample before testing using the procedure in this table for oxidizing agents.
Metals	Nickel or cobalt in concentrations up to 1 mg/L do not interfere. Eliminate the interference from up to 20 mg/L copper and 5 mg/L iron by adding the contents of one HexaVer Chelating Reagent Powder Pillow (Cat. No. 243-99) to the sample and then mixing before adding the CyaniVer 3 Cyanide Reagent Powder Pillow in step 3. Prepare a reagent blank of deionized water and reagents to zero the instrument in step 13.
Oxidizing Agents	**1.** Adjust a 25-mL portion of the alkaline sample to pH 7–9 with 2.5 N Hydrochloric Acid Standard Solution (Cat. No. 1418-32). Count the number of drops of acid added. **2.** Add two drops of Potassium Iodide Solution (Cat. No. 343-32) and two drops of Starch Indicator Solution (Cat. No. 349-32) to the sample. Swirl to mix. The sample will turn blue if oxidizing agents are present. **3.** Add Sodium Arsenite Solution (Cat. No. 1047-32) drop-wise until the sample turns colorless. Swirl the sample thoroughly after each drop. Count the number of drops. **4.** Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in step a. Subtract one drop from the amount of Sodium Arsenite Solution added in step c. Add this amount to the sample and mix thoroughly. Continue with step 3 of the cyanide procedure.

(continued)

Interfering Substance	Interference Levels and Treatments
Reducing Agents	**1.** Adjust a 25-mL portion of the alkaline sample to pH 7–9 with 2.5 N Hydrochloric Acid Standard Solution. (Cat. No. 1418-32) Count the number of drops added. **2.** Add four drops of Potassium Iodide Solution (Cat. No. 343-32) and four drops of Starch Indicator Solution to the sample. Swirl to mix. The sample should be colorless. **3.** Add Bromine Water (Cat. No. 2211-20) drop-wise until a blue color appears. Swirl the sample thoroughly after each addition. Count the number of drops. **4.** Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in step a. **5.** Add the total number of drops of Bromine Water counted in *step c* to the sample and mix thoroughly. Continue with *step 3* of the cyanide procedure.
Turbidity	Large amounts of turbidity will cause high readings. Use filter paper (Cat. No. 1894-57) and a funnel (Cat. No. 1083-67) to filter highly turbid water samples before use in *steps 2* and *11*. The test results should then be recorded as soluble cyanide.

Sample Collection, Storage and Preservation

Collect samples in glass or plastic bottles and analyze as quickly as possible.

The presence of oxidizing agents, sulfides and fatty acids can cause the loss of cyanide during sample storage. Samples containing these substances must be pretreated as described below before preservation with sodium hydroxide. If the sample contains sulfide and is not pretreated, it must be analyzed within 24 hours.

Preserve the sample by adding 4.0 mL of 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53) to each liter (or quart) of sample, using a glass serological pipet and pipet filler. Check the sample pH; 4-mL of sodium hydroxide is usually enough to raise the pH of most water and wastewater samples to 12. Add more 5.0 N Sodium Hydroxide if necessary. Store the samples at 4 °C (39 °F) or less. Samples preserved in this manner can be stored for 14 days.

Before testing, samples preserved with 5.0 N Sodium Hydroxide or samples that are highly alkaline due to chlorination treatment processes or sample distillation procedures should be adjusted to approximately pH 7 with 2.5 N Hydrochloric Acid Standard Solution. Where significant amounts of preservative are used, a volume correction should be made; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Oxidizing Agents

Oxidizing agents such as chlorine decompose cyanides during storage. To test for their presence and to eliminate their effect, pretreat the sample as follows:

a. Take a 25-mL portion of the sample and add one drop of 10-g/L m-Nitrophenol Indicator Solution (Cat. No. 2476-32). Swirl to mix.

b. Add 2.5 N Hydrochloric Acid Standard Solution drop-wise until the color changes from yellow to colorless. Swirl the sample thoroughly after the addition of each drop.

c. Add two drops of Potassium Iodide Solution, 30-g/L (Cat. No. 343-32), and two drops of Starch Indicator Solution, to the sample. Swirl to mix. The solution will turn blue if oxidizing agents are present.

d. If step 3 suggests the presence of oxidizing agents, add two level, 1-g measuring spoonfuls of Ascorbic Acid (Cat. No. 6138-26) per liter of sample.

e. Withdraw a 25-mL portion of sample treated with ascorbic acid and repeat *steps 1* to *3*. If the sample turns blue, repeat *steps 4* and *5*.

f. If the 25-mL sample remains colorless, preserve the remaining sample to pH 12 for storage with 5 N Sodium Hydroxide Standard Solution (usually 4-mg/L) (Cat. No. 2450-53).

g. Perform the procedure given under *Interferences*, *Reducing Agents*, to eliminate the effect of excess ascorbic acid, before following the cyanide procedure.

Sulfides

Sulfides will quickly convert cyanide to thiocyanate (SCN^-). To test for the presence of sulfide and eliminate its effect, pretreat the sample as follows:

a. Place a drop of sample on a disc of Hydrogen Sulfide Test Paper (Cat. No. 25377-33) that has been wetted with pH 4 Buffer Solution (Cat. No. 12223-49).

b. If the test paper darkens, add a 1-g measuring spoon of Lead Acetate to the sample. Repeat *step a*.

c. If the test paper continues to turn dark, keep adding Lead Acetate (Cat. No. 7071-34) until the sample tests negative for sulfide.

d. Filter the lead sulfide precipitate through Filter Paper (Cat. No. 1894-57) and a Funnel (Cat. No. 1083-67). Preserve the sample for storage with 5 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53) or neutralize to a pH of 7 for analysis.

Fatty Acids
Caution: Perform this operation in a hood and as quickly as possible.

When distilled, fatty acids will pass over with cyanide and, under the alkaline conditions of the absorber, will form soaps. If the presence of fatty acid is suspected, use the following pretreatment before preserving samples with sodium hydroxide.

a. Acidify 500 mL of sample to pH 6 or 7 with a 4:1 dilution of glacial Acetic Acid (Cat. No. 100-49).

b. Pour the sample into a 1000-mL separatory funnel and add 50 mL of Hexane (Cat. No.14478-49).

c. Stopper the funnel and shake for one minute. Allow the layers to separate.

d. Drain off the lower, sample layer into a 600-mL beaker. If the sample is to be stored, add enough 5 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53) to raise the pH above 12.

Acid Distillation

All samples to be analyzed for cyanide should be treated by acid distillation except when experience has shown that there is no difference in results obtained with or without distillation. With most compounds, a one-hour reflux is adequate.

If thiocyanate is present in the original sample, a distillation step is absolutely necessary as thiocyanate causes a positive interference. High concentrations of thiocyanate can yield a substantial quantity of sulfide in the distillate. The "rotten egg" smell of hydrogen sulfide will accompany the distillate when sulfide is present. The sulfide must be removed from the distillate prior to testing.

If cyanide is not present, the amount of thiocyanate can be determined. The sample is not distilled and the final reading is multiplied by 2.2. The result is mg/L SCN^-.

The distillate can be tested and treated for sulfide after the last step of the distillation procedure by using the following lead acetate treatment procedure.

a. Place a drop of the distillate (already diluted to 250 mL) on a disc of Hydrogen Sulfide Test Paper (Cat. No. 25377-33) that has been wetted with pH 4.0 Buffer Solution (Cat. No. 12223-49).

b. If the test paper darkens, add 2.5 N Hydrochloric Acid Standard Solution (Cat. No. 1418-32) drop-wise to the distillate until a neutral pH is obtained.

c. Add a 1-g measuring spoon of lead acetate (Cat. No. 7071-34) to the distillate and mix. Repeat step 1.

d. If the test paper continues to turn dark, keep adding lead acetate until the distillate tests negative for sulfide.

e. Filter the black lead sulfide precipitate through filter paper (Cat. No. 1894-57) and a funnel (Cat. No. 1083-67). Neutralize the liquid filtrate to pH 7 and immediately analyze for cyanide.

Distillation Procedure

The following steps describe the distillation process using distillation apparatus (Cat. No. 27744-00 or -02 for 115 VAC or 230 VAC, respectively) and cyanide glassware (Cat. No. 22658-00) offered by Hach:

a. Set up the distillation apparatus for cyanide recovery, leaving off the thistle tube. Refer to the *Hach Distillation Apparatus Manual*. Turn on the water and make certain it is flowing steadily through the condenser.

b. Fill the distillation apparatus cylinder to the 50-mL mark with 0.25 N Sodium Hydroxide Standard Solution (Cat. No. 14763-53).

c. Fill a clean 250-mL graduated cylinder to the 250-mL mark with sample and pour it into the distillation flask. Place a stirring bar into the flask and attach the thistle tube.

d. Arrange the vacuum system as shown in the Hach Distillation Apparatus Manual, but do not connect the vacuum tubing to the gas bubbler. Turn on the water to the aspirator to full flow and adjust the flow meter to 0.5 SCFH.

e. Connect the vacuum tubing to the gas bubbler, making certain that air flow is maintained (check the flow meter) and that air is bubbling from the thistle tube and the gas bubbler.

f. Turn the power switch on and set the stir control to 5. Using a 50-mL graduated cylinder, pour 50 mL of 19.2 N Sulfuric Acid Standard Solution (Cat. No. 2038-49) through the thistle tube and into the distillation flask.

g. Using a water bottle, rinse the thistle tube with a small amount of deionized water.

h. Allow the solution to mix for three minutes; then add 20 mL Magnesium Chloride Reagent (Cat. No. 14762-53) through the thistle tube and rinse again. Allow the solution to mix for 3 more minutes.

i. Verify that there is a constant flow of water through the condenser.

j. Turn the heat control to 10.

k. Carefully monitor the distillation flask at this point in the procedure. Once the sample begins to boil, slowly lower the air flow to 0.3 SCFH. If the contents of the distillation flask begin to back up through the thistle tube, increase the air flow by adjusting the flow meter until the contents do not back up through the thistle tube. Boil the sample for one hour.

l. After one hour, turn off the still, but maintain the air flow for 15 minutes more.

m. After 15 minutes, remove the rubber stopper on the 500-mL vacuum flask to break the vacuum and turn off the water to the aspirator. Turn off the water to the condenser.

n. Remove the gas bubbler/cylinder assembly from the distillation apparatus. Separate the gas bubbler from the cylinder and pour the contents of the cylinder into a 250-mL, Class A volumetric flask. Rinse the gas bubbler, cylinder and J-tube connector with deionized water and add the washings to the volumetric flask.

o. Fill the flask to the mark with deionized water and mix thoroughly. Neutralize the contents of the flask and analyze for cyanide.

Calibration Standard Preparation

To perform a cyanide calibration using the Pyridine-Pyrazalone method, prepare calibration standard containing 0.05, 0.100, and 0.200 mg/L cyanide as follows:

a. Prepare a 100-mg/L cyanide stock solution as described in the *Accuracy Check*.

b. Into three different 1000-mL Class A volumetric flasks, pipet 0.50, 1.00, and 2.00 mL of the 100-mg/L cyanide stock solution, respectively. Use Class A pipets.

c. Dilute each flask to volume with deionized water. Stopper and invert several times to mix.

d. Using the Pyridine-Pyrazalone method and the calibration procedure described in the User-Entered Programs section of the *Instrument Manual*, generate a calibration curve from the standards prepared above.

Accuracy Check

Standard Solutions Method

Caution: Cyanides and their solutions, and the hydrogen cyanide liberated by acids, are very poisonous. Both the solutions and the gas can be absorbed through the skin.

Prepare a 100 mg/L cyanide stock solution weekly as follows:

1. Dissolve 0.2503 grams or an equivalent amount of pure potassium cyanide in deionized water and dilute to 1000 mL.

2. Immediately before use, prepare a 0.200 mg/L cyanide working solution by diluting 2.00 mL of the 100 mg/L stock solution to 1000 mL using deionized water.
3. To adjust the calibration curve using the reading obtained with the 0.200 mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected units). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.120 mg/L CN^-

Program	95% Confidence Limits of Distribution
160	0.110–0.130 mg/L CN^-

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.001 mg/L CN^-

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The Pyridine-Pyrazalone method used for measuring cyanide gives an intense blue color with free cyanide. A sample distillation is required to determine cyanide from transition and heavy metal cyanide complexes. Test results are measured at 612 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Cyanide Reagent Set			24302-00
Includes:			
(1) CyaniVer® 3 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21068-69
(1) CyaniVer® 4 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21069-69
(1) CyaniVer® 5 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21070-69

Required Apparatus

Cylinder, graduated, 10-mL	1	each	508-38
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Potassium Cyanide, ACS	125 g	767-14
Water, deionized	4 liters	272-56

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Cyanide

Cyanogen Chloride*

(0.01 to 0.50 mg/L CN^-)

UniCell™ Vials

Scope and Application: For wastewater process control

* Reagent sets for this method are only available in Europe.

Tips and Techniques

- Analyze samples collected in glass or plastic bottles as quickly as possible.
- Adjust pH of preserved samples to between pH 7–10 with 1.0 N Sulfuric Acid Standard Solution before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check*.
- See *Section 3.2.4* on page *40* in the procedure manual for information on adjusting the calibration curve.
- The shelf life of test reagents can be extended to 24 months if kept at 4 °C.
- Make sure that the temperature of the water sample and the sample vial is between 15–25 °C (59–77 °F).
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell™ Vials

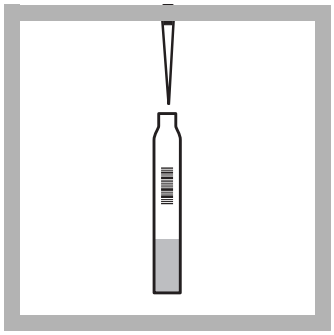
1. Touch

Hach Programs.

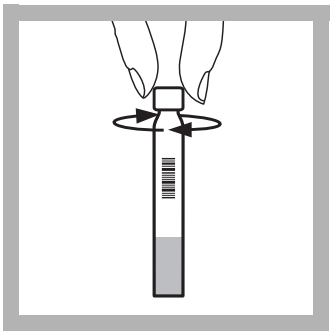
Select program

813 Cyanide, HCT 129

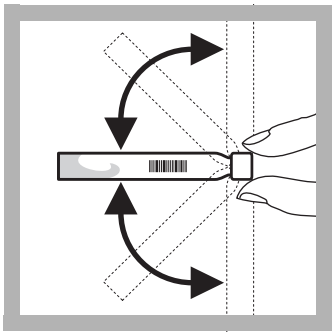
Touch **Start**.



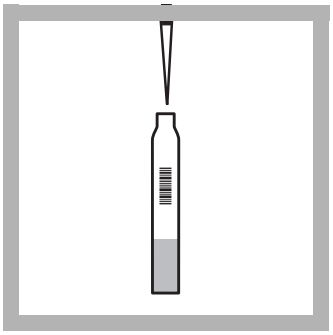
2. Pipet 2 mL of sample into a sample vial.



3. Cap with **grey** UniCap A (HCT 129 A).

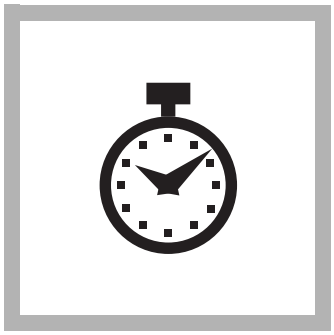


4. Invert the sample vial repeatedly until the solids in the cap dissolve completely.



5. Pipet 2 mL of Pyridine-hydrochloric acid B (HCT 129 B) into the sample vial.

Recap the vial. Invert several times to mix.

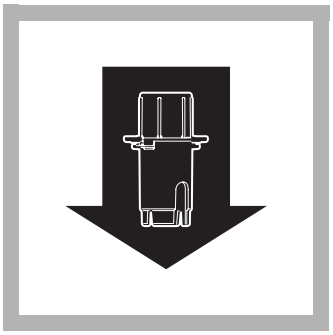


6. Touch the timer icon.

Touch **OK**.

An 8-minute reaction period will begin.

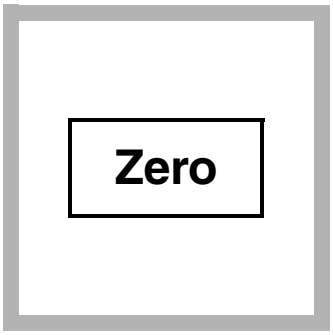
After the reaction period, invert the sample vial again to mix completely.



7. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

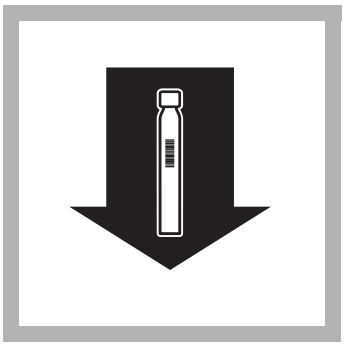
When the timer beeps, wipe the zero vial (**white** cap) and place it into the cell adapter.



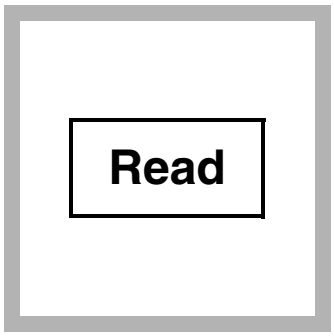
8. Touch **Zero**.

The display will show:

0.00 mg/L CN^-
Underrange



9. Wipe the sample vial and place it into the celladapter.



10. Touch **Read**.

Results will appear in mg/L CN^-.

Interferences

Interferences are caused by substances like formaldehyde which react with the cyanide. Reducing agents (like sulfite) and other compounds that affect the reactivity of chlorine also interfere. Thiocyanate and cyanide react similarly with chlorine to form cyanogen chloride, thus the thiocyanate will be a positive interference in the test.

Sample Collection, Preservation, and Storage

Samples collected in glass or plastic containers should be analyzed as quickly as possible. Preserve the sample by adjusting the pH higher than 12 by adding 5.0 N Sodium Hydroxide Standard Solution (4 mL per 1000 mL). Store the samples at 4 °C (39 °F) or less.

Accuracy Check

Standard Additions Method (Sample Spike)
Caution: cyanides and their solutions, and the hydrogen cyanide liberated by acids, are very poisonous. Both the solutions and the gas can be absorbed through the skin.

1. Prepare a 100-mg/L CN^- stock solution weekly by dissolving 0.2503 grams, or an equivalent amount of pure potassium cyanide, in deionized water and diluting to 1000 mL.
2. Immediately before use, prepare a 10.00-mg/L cyanide working solution by diluting 10.00 mL of the 100-mg/L stock solution to 100 mL using deionized water.
3. After reading test results, leave the sample cell (unspiked sample) in the instrument.
4. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
5. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
6. Measure 100 mL of sample into three graduated cylinders.
7. Pipet 0.5 mL, 1.0 mL, and 1.5 mL of 10-mg/L CN^-, respectively, into the three cylinders and mix thoroughly.
8. Analyze as described in the procedure. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
9. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Analyze a 0.30-mg/L CN^- Standard Solution using the procedure described above.
2. To adjust the calibration curve using the reading obtained with the 0.200-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Perform the cyanide procedure as described above. See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.30 mg/L Free CN^-

Program	95% Confidence Limits of Distribution
813	0.23–0.37 mg/L CN^-

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
813	Entire range	0.010	0.01 mg/L CN^-

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Cyanides react with chlorine to form cyanogen chloride, which in turn reacts with pyridine in the presence of barbituric acid, condensing to form a violet-colored compound. Measurements are taken at 588 nm.

Required Reagents

Description	Unit	Cat. No.
Cyanide - CN, UniCell™ HCT 129*	23/pkg	HCT 129

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Flask, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 100-mL	each	20886-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pk/100	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	pk/400	27950-00
pH Paper	pk/100	26013-00

Optional Reagents

Potassium Cyanide	125 g	767-14
Sulfuric Acid Standard Solution, 1.00 N	1 L	1270-53

* Available in Europe only

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Cyanuric Acid

Method 8139

Powder Pillows

Scope and Application: For water

Turbidimetric Method

(5 to 50 mg/L)

Tips and Techniques

- Filter highly turbid samples with filter paper (Cat. No. 1894-57) and a funnel (Cat. No.1083-67).
- After adding the reagent, a white turbidity will form if cyanuric acid is present.
- Clean sample cells with soap, water, and a brush soon after each test to avoid a build-up of film on the sample cell.

Powder Pillows — Method 8139

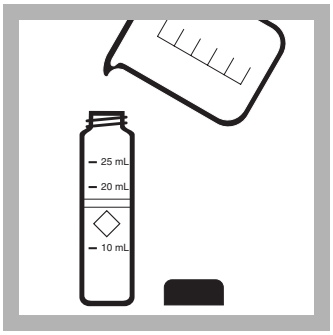
1. Touch

Hach Programs.

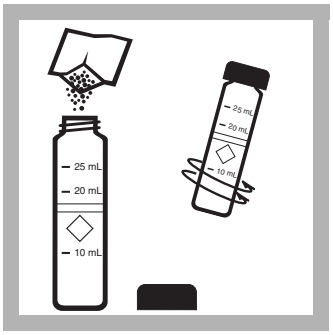
Select program

170 Cyanuric Acid.

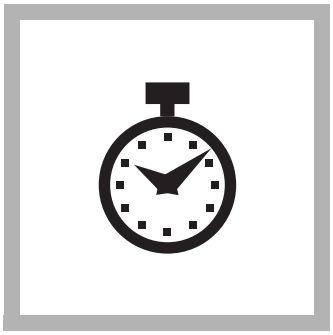
Touch **Start**.



2. Fill a round sample cell with 25 mL of sample.



3. Add the contents of one Cyanuric Acid 2 Reagent Powder Pillow (the prepared sample). Swirl to mix.

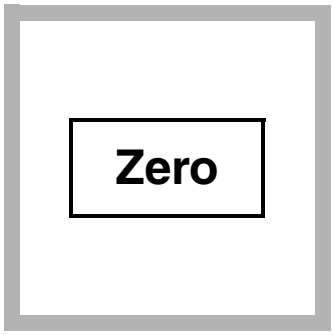


4. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.

5. Fill a second sample cell with 25 mL of sample (the blank) and place it in the cell holder.

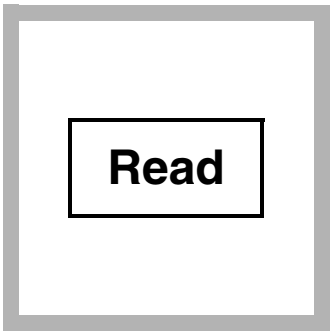


6. Touch **Zero**.

The display will show:

0 mg/L Cyan Acid

7. Within seven minutes after the timer beeps, place the prepared sample into the cell holder.



8. Touch **Read**.

Results will appear in mg/L Cyan Acid.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Samples must be analyzed within 24 hours.

Accuracy Check

Standard Solution Method

Dissolve 1.000 gram of cyanuric acid in l liter of deionized water to make a 1000-mg/L solution. Cyanuric acid is difficult to dissolve; it may take several hours to completely dissolve. This solution is stable for several weeks.

Dilute 3.00 mL of the 1000-mg/L solution to 100 mL with deionized water to make a 30-mg/L solution. Prepare fresh daily.

Testing the 30-mg/L solution should give test results of about 30 mg/L cyanuric acid.

Standard Curve Adjustment

Use the standard prepared in the Accuracy Check. Adjust the calibration curve using the reading obtained with the 30-mg/L standard solution:

1. Touch **Options** on the current program menu. Touch **Adjustments & Accuracy** on the Options menu. Touch **Standard Adjust.**
2. Touch **On**. Touch **OK** to accept the displayed concentration. If an alternated concentration is used, touch the number in the **Adjust to** field and enter the actual concentration. Press **OK.**

Method Performance

Precision

Standard: 10 mg/L cyanuric acid

Program	95% Confidence Limits of Distribution
170	7–13 mg/L cyanuric acid

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
10 mg/L	0.010	0.3 mg/L cyanuric acid
30 mg/L	0.010	0.3 mg/L cyanuric acid
50 mg/L	0.010	0.4 mg/L cyanuric acid

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The test for Cyanuric Acid uses the turbidimetric method. Cyanuric Acid 2 Reagent precipitates any Cyanuric Acid present and holds it in suspension. The amount of turbidity caused by the suspended particles is directly proportional to the amount of cyanuric acid present. Test results are measured at 480 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Cyanuric Acid 2 Reagent Powder Pillow	1 pillow	50/pkg	2460-66

Required Apparatus

Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Cyanuric Acid	25 g	7129-24
Water, deionized	4 L	272-56

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3/04 1ed

★Method 8029 SPADNS Method*

Reagent Solution or AccuVac® Ampuls (0.02 to 2.00 mg/L F⁻)

Scope and Application: For water, wastewater and seawater; USEPA accepted for reporting for drinking and wastewater analyses (distillation required; see *Distillation on page 3*)**

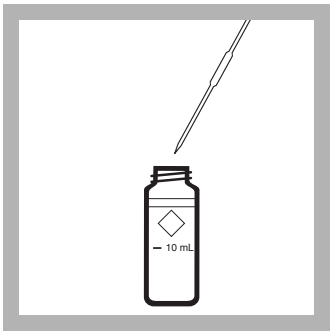
* Adapted from *Standard Methods for the Examination of Water and Wastewater, 4500-F B & D*
** Procedure is equivalent to USEPA method 340.1 for drinking water and wastewater.

Tips and Techniques

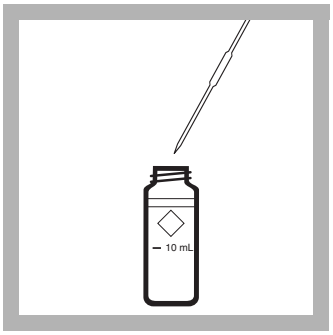
- The sample and deionized water should be at the same temperature (±1 °C). Temperature adjustments may be made before or after reagent addition.
- SPADNS Reagent is toxic and corrosive. Use care while handling the reagent.
- For best results, measure the volume of SPADNS Reagent as accurately as possible.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- SPADNS Reagent contains sodium arsenite. Final solutions will contain arsenic (D004) in sufficient concentration to be regulated as a hazardous waste for Federal RCRA. See *Section 4* for information on proper disposal of these materials.
- If the instrument displays **OVER!**, dilute a fresh sample (5 mL) with an equal volume of deionized water and repeat the test, using this solution in *step 2*. Multiply the result by 2.

Using SPADNS Reagent Method 8029

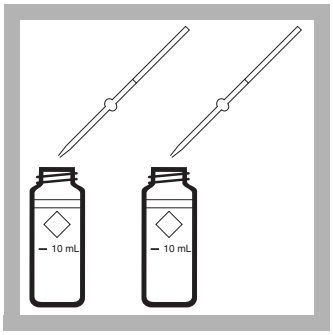
1. Touch **Hach Programs**.
Select program **190 Fluoride**.
Touch **Start**.



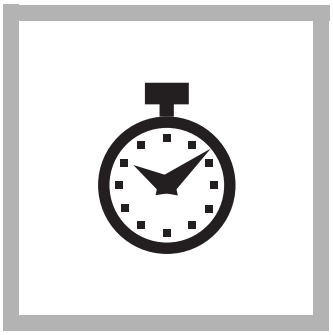
2. Pipet 10.0 mL of sample into a dry, round sample cell (this is the prepared sample).



3. Pipet 10.0 mL of deionized water into a second dry, round sample cell (this is the blank).



4. Carefully pipet 2.0 mL of SPADNS Reagent into each cell. Swirl to mix.

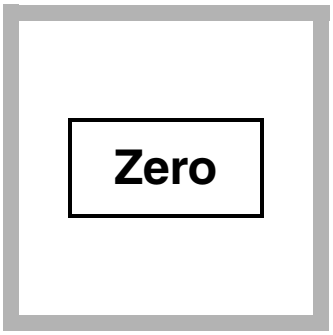


5. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L F^-

8. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L F^-.

AccuVac Ampul — Method 8029

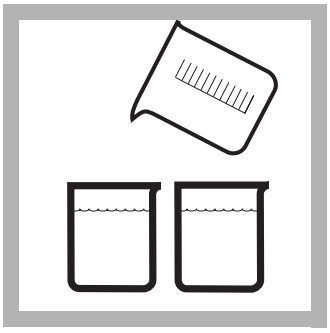
1. Touch

Hach Programs.

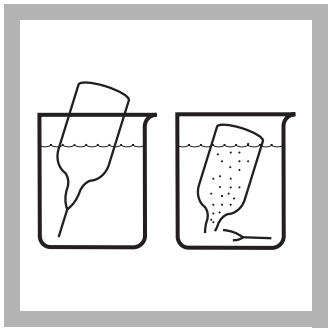
Select program

195 Fluoride AV.

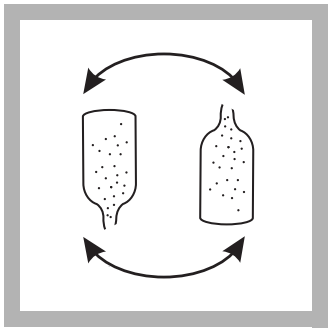
Touch **Start**.



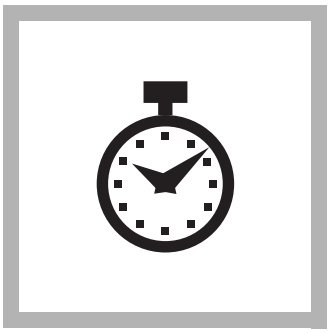
2. Collect at least 40 mL of sample in a 50-mL beaker. Pour at least 40 mL of deionized water into a second beaker.



3. Fill one SPADNS Fluoride Reagent AccuVac Ampul with sample. Fill another ampule with deionized water (the blank). Keep the tips immersed while the ampules fill completely.



4. Quickly invert the ampules several times to mix. Wipe off any liquid or fingerprints.

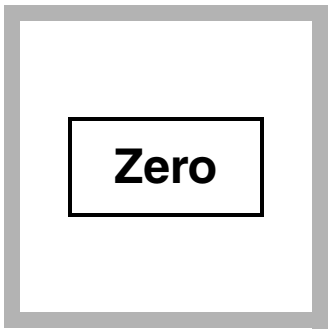


5. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L F^-

8. Place the AccuVac Ampul that contains the sample into the cell holder.

Touch **Read**.

Results will appear in mg/L F^-.

Interferences

This test is sensitive to small amounts of interference. Glassware must be very clean (acid rinse before each use). Repeat the test with the same glassware to ensure that results are accurate.

Interfering Substance	Interference Levels and Treatments
Alkalinity (as $CaCO_3$)	At 5000 mg/L it causes a −0.1 mg/L F^- error.
Aluminum	At 0.1 mg/L it causes a −0.1 mg/L F^- error. To check for interferences from aluminum, read the concentration one minute after reagent addition, then again after 15 minutes. An appreciable increase in concentration suggests aluminum interference. Waiting 2 hours before making the final reading will eliminate the effect of up to 3.0 mg/L aluminum.
Chloride	At 7000 mg/L it causes a +0.1 mg/L F^- error.
Chlorine	SPADNS Reagent contains enough arsenite to eliminate interference up to 5 mg/L chlorine. For higher chlorine levels, add one drop of Sodium Arsenite Solution (Cat. No. 1047-32) to 25 mL of sample for each 2 mg/L of Chlorine.
Iron, ferric	At 10 mg/L it causes a −0.1 mg/L F^- error.
Phosphate, ortho	At 16 mg/L it causes a +0.1 mg/L F^- error.
Sodium Hexameta-phosphate	At 1.0 mg/L it causes a +0.1 mg/L F^- error.
Sulfate	At 200 mg/L it causes a +0.1 mg/L F^- error.

Distillation

1. Most interferences can be eliminated by distilling the sample from an acid solution as described below:
2. Set up the distillation apparatus for general purpose distillation. Refer to the Distillation Apparatus manual for proper assembly. Use a 125-mL Erlenmeyer flask to collect the distillate.

3. Turn on the water and maintain a steady flow through the condenser.
4. Measure 100 mL of sample into the distillation flask using a 100-mL graduated cylinder. Add a magnetic stir bar and 5 glass beads.
5. For proof of accuracy, use a 1.0-mg/L Fluoride Standard Solution in place of the sample.
6. Turn the stirrer power switch on. Turn the stir control to 5.
7. Using a 250-mL graduated cylinder, carefully add 150 mL of StillVer® Distillation Solution into the flask. (StillVer Distillation Solution is a 2:1 mixture of concentrated sulfuric acid and water.)

Note: *When distilling samples with high amounts of chloride, add 5 mg of Silver Sulfate (Cat. No. 334-14) to the sample for every mg/L of chloride in the sample.*

8. With the thermometer in place, turn the heat control to 10. The yellow pilot lamp indicates the heater is on.
9. When the temperature reaches 180 °C or when 100 mL of distillate has been collected, turn the still off (requires about 1 hour).
10. Dilute the distillate to a volume of 100 mL, if necessary. The distillate may now be analyzed by the SPADNS or the fluoride ion-selective electrode method.

Sample Collection, Storage and Preservation

Samples may be stored in glass or plastic bottles for at least seven days when cooled to 4 °C (39 °F) or lower. Warm samples to room temperature before analysis.

Accuracy Check

Standard Solution Method

A variety of standard solutions covering the entire range of the test is available from Hach. Use these in place of sample to verify technique.

Minor variations between lots of reagent become measurable above 1.5 mg/L. While results in this region are usable for most purposes, better accuracy may be obtained by diluting a fresh sample 1:1 with deionized water and retesting. Multiply the result by 2.

To adjust the calibration curve using the reading obtained with a Fluoride Standard Solution:

1. Touch **Options** on the current program menu. Touch **Standard Adjust: Off**.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected units). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 1.00 mg/L F^-

Program	95% Confidence Limits of Distribution
190	0.95–1.05 mg/L F^-
195	0.88–1.12 mg/L F^-

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Sensitivity	ΔAbs	ΔConcentration (Program 190)	ΔConcentration (Program 195)
at 0	0.010	0.03 mg/L F^-	0.02 mg/L F^-
at 1 ppm	0.010	0.03 mg/L F^-	0.03 mg/L F^-
at 2 ppm	0.010	0.04 mg/L F^-	0.04 mg/L F^-

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The SPADNS Method for fluoride determination involves the reaction of fluoride with a red zirconium-dye solution. The fluoride combines with part of the zirconium to form a colorless complex, thus bleaching the red color in an amount proportional to the fluoride concentration. This method is accepted by the EPA for NPDES and NPDWR reporting purposes when the samples have been distilled. Seawater and wastewater samples require distillation. Test results are measured at 580 nm.

Fluoride

Required Reagents

Description	Quantity Required per Test	Unit	Cat. No.
SPADNS Reagent Solution	4 mL	500 mL	444-49
or SPADNS Fluoride Reagent AccuVac® Ampuls	2 ampuls	25/pkg	25060-25
Water, deionized	10 mL	4 liters	272-56

Required Apparatus (Using Solution)

Pipet Filler safety bulb	1	each	14651-00
Pipet, volumetric, Class A, 2.00-mL	1	each	14515-36
Pipet, volumetric, Class A, 10.00-mL	1	each	14515-38
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Thermometer, -10 to 110 °C	1	each	1877-01

Required Apparatus (Using AccuVac® Ampuls)

Beaker, 50-mL	2	each	500-41H

Required Standards

Fluoride Standard Solution, 0.2-mg/L F^-		500 mL	405-02
Fluoride Standard Solution, 0.5-mg/L F^-		500 mL	405-05
Fluoride Standard Solution, 0.8-mg/L F^-		500 mL	405-08
Fluoride Standard Solution, 1.0-mg/L F^-		1000 mL	291-53
Fluoride Standard Solution, 1.0-mg/L F^-		500 mL	291-49
Fluoride Standard Solution, 1.2-mg/L F^-		500 mL	405-12
Fluoride Standard Solution, 1.5-mg/L F^-		500 mL	405-15
Fluoride Standard Solution, 2.0-mg/L F^-		500 mL	405-20
Fluoride Standard Solution, 100-mg/L F^-		500 mL	232-49

Distillation Reagents and Apparatus

Cylinder, graduated, 100-mL	1	each	508-42
Cylinder, graduated, 250-mL	1	each	508-46
Select one:			
Distillation Heater and Support Apparatus Set, 115 VAC, 50/60 Hz	1	each	22744-00
Distillation Heater and Support Apparatus Set, 230 VAC, 50/60 Hz	1	each	22744-02
Distillation Apparatus Set, General Purpose	1	each	22653-00
Flask, Erlenmeyer, 125-mL	1	each	20897-43
Glass Beads	5	100/pkg	2596-00
StillVer® Distillation Solution		500 mL	446-49
Stir Bar, magnetic	1	1	10764-16

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Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Fluoride

SPADNS

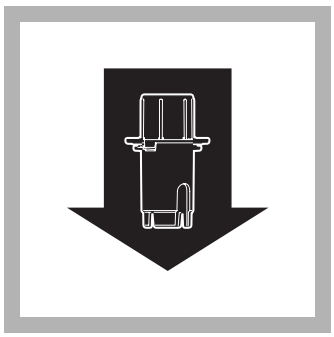
UniCell™ Vials (0.10 to 1.50 mg/L F⁻)

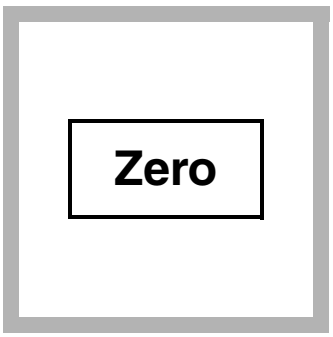
Scope and Application: For drinking water, ground water, surface water, wastewater, and process control

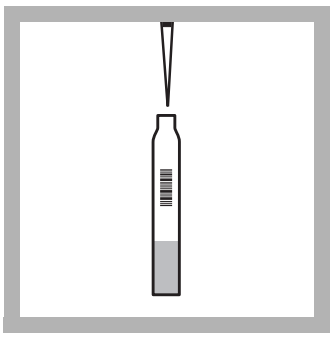
Tips and Techniques

- Analyze samples collected in acid-cleaned glass or plastic bottles as quickly as possible.
- Adjust the pH of the sample to between 1–11 with hydrochloric acid before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check*.
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is between 15–25 °C.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials







1. Touch

Hach Programs.

Select program

810 Fluoride, HCT 132

Touch **Start**.

2. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

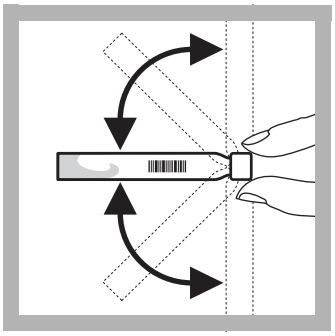
Wipe an empty sample vial and place it into the cell adapter.

3. Touch **Zero**.

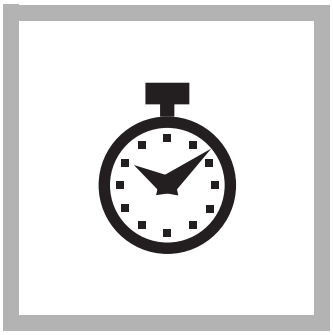
The display will show:

0.00 mg/L F⁻
Underrange

4. Remove the sample vial from the instrument, uncap the vial, and pipet 5.0 mL of sample into it.



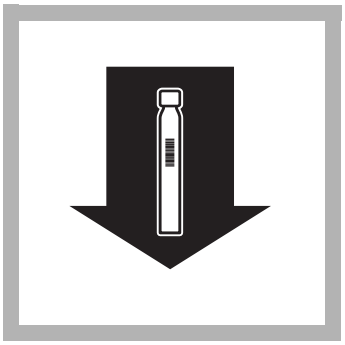
5. Cap the sample vial and invert several times to mix.



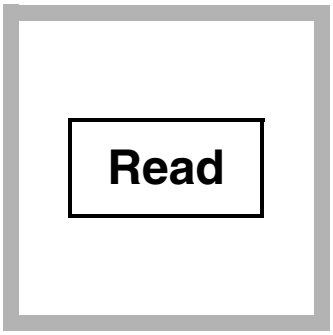
6. Touch the timer icon.

Touch **OK**.

A 1-minute reaction period will begin.



7. When the timer beeps, wipe the sample vial and place it into the celladapter.



8. Touch **Read**.

Results will appear in mg/L F^-.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Cl^-	7000 mg/L
SO_4^{2-}	200 mg/L
Mn^{2+}	30 mg/L
PO_4^{3-}	16 mg/L
Fe^{2+}, Fe^{3+}	10 mg/L
Cl_2	3 mg/L
Sodium metaphosphate	1 mg/L
Al^{3+}	0.1 mg/L

Sample Collection, Preservation, and Storage

Collect samples in acid-cleaned glass or plastic containers. Store the samples at 4 °C (39 °F) or less, for up to 28 days.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.

3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Measure 100 mL of sample into three graduated cylinders.
5. Pipet 0.20 mL, 0.40 mL, and 0.60 mL of 100-mg/L F^- Standard Solution, respectively, into the three cylinders and mix thoroughly.
6. Analyze as described in the procedure. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Analyze a 1.00-mg/L F^- Standard Solution using the procedure described above.
2. To adjust the calibration curve using the reading obtained with the 1.00-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Perform the Fluoride procedure as described above. See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 1.00 mg/L F^-

Program	95% Confidence Limits of Distribution
810	0.76–1.24 mg/L F^-

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
810	0.30	0.010	0.05 mg/L F^-
810	0.80	0.010	0.06 mg/L F^-
810	1.20	0.010	0.06 mg/L F^-

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Fluoride ions react with a red zirconium complex to form a colorless zirconium fluoride complex. The color fades in proportion to the amount of fluoride present in the solution. Measurements are taken at 588 nm.

Required Reagents

Description	Unit	Cat. No.
Fluoride - F, UniCell™ HCT 132	24/pkg	HCT 132

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Graduated cylinder, mixing, 100-mL	each	20886-42
Flask, volumetric 100 mL	each	14574-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pkg/100	27952-00
Pipettor, (Jencons) 100–1000 µL	1 each	27949-00
Replacement tips for 27949-00	pkg/400	27950-00
pH Paper	pkg/100	26013-00

Optional Reagents

Fluoride Standard Solution, 1.00-mg/L F	200 mL	291-29
Fluoride Standard Solution, 100-mg/L F	500 mL	232-49

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3/04 1ed

Formaldehyde

Method 8110

MBTH Method*

Powder Pillows

(3 to 500 µg/L)

Scope and Application: For water

* Adapted from Matthews, T.G. and Howell, T.C., *Journal of the Air Pollution Control Association*, 31 (11) 1181-1184 (1981).

Tips and Techniques

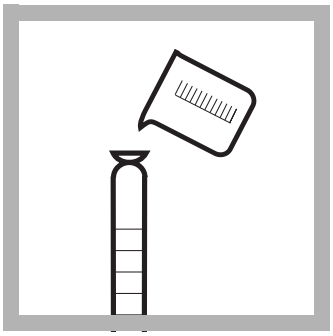
- Analyze samples immediately. Do not preserve for later analysis.
- Wash glassware with Chromic Acid Cleaning Solution (Cat. No. 1233-49) to remove trace contaminants.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Time and temperature are very important in this test. The sample should be 25 ±1°C, and the times specified in steps must be followed precisely. A temperature-controlled water bath is recommended for best accuracy.
- Obtain formaldehyde-free water by distilling water from alkaline permanganate (4 g Sodium Hydroxide (Cat. No. 187-34), 2 g Potassium Permanganate (Cat. No. 769-05) per 500 mL of water). Discard the first 50–100 mL of distillate.

Powder Pillows — Method 8110

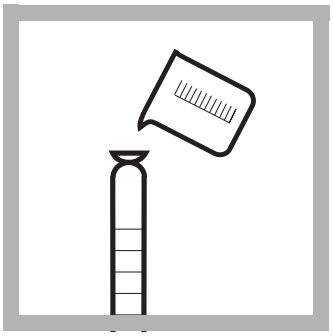
1. Touch **Hach Programs**.

Select program **200 Formaldehyde**.

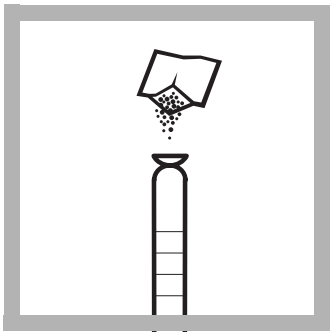
Touch **Start**.



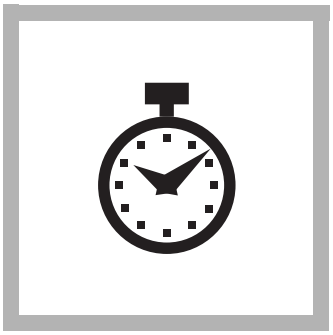
2. Accurately measure 25 mL of sample in a 50-mL mixing cylinder (this is the prepared sample).



3. Accurately measure 25 mL of formaldehyde-free water in a second 50-mL mixing cylinder (this is the blank).



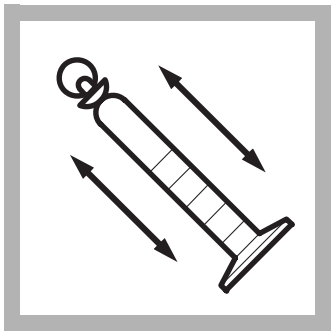
4. Add the contents of one MBTH Powder Pillow to the blank. Stopper the cylinder.



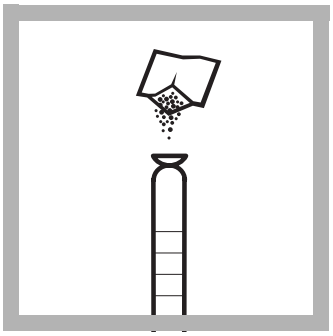
5. Immediately touch the timer icon.

A 17-minute reaction period will begin. Proceed with step 6 immediately after the timer starts.

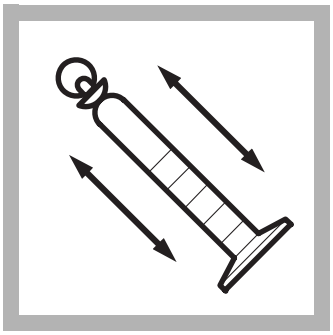
Complete steps 6–11 during the reaction period, at the times specified.



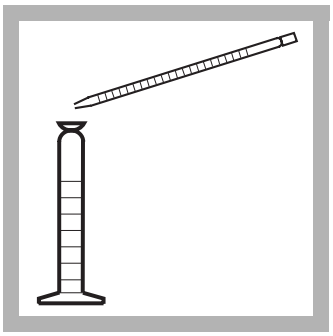
6. Immediately after the reaction period starts, shake the cylinder (the blank) vigorously for 20 seconds. Do not wait for the timer to beep.



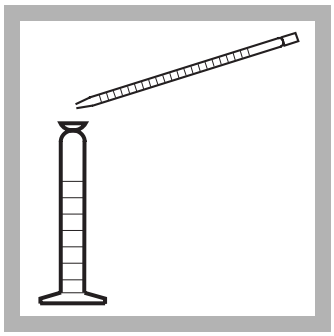
7. Add the contents of one MBTH Powder Pillow to the prepared sample when the timer displays **15:00**.



8. Stopper the cylinder and shake vigorously for 20 seconds.



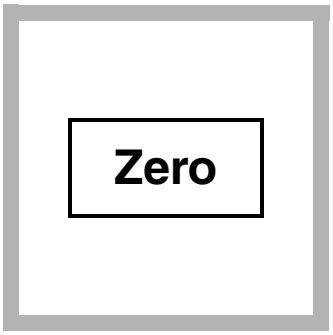
9. Add 2.5 mL of Developing Solution for Low Range Formaldehyde to the blank when the timer shows **12:00**. Stopper and invert to mix.



10. Add 2.5 mL of Developing Solution for Low Range Formaldehyde to the prepared sample when the timer shows **10:00**. Stopper and invert to mix.

11. Just before the timer shows **2:00**, pour the blank into the sample cell. Pour the solution slowly to avoid bubble formation on the cell walls. If bubbles form, swirl to dislodge them.

12. Immediately wipe the blank and place it into the cell holder.



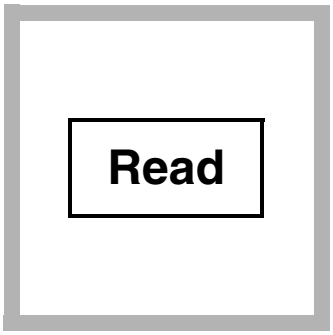
13. When the timer shows **2:00**, touch **Zero**.

The display will show:

0 µg/L CH_2O

14. Pour the prepared sample into a sample cell. Wipe the cell and place it into the cell holder.

15. Wipe the cell and place it into the cell holder.



16. When the timer beeps, touch **Read**.

Results will appear in µg/L CH_2O.

Interferences

Interfering Substance	Interference Levels and Treatments
Acetate	Greater than 1000 mg/L
Aldehydes (other)	Positive interference at all levels
Ammonium (as N)	Greater than 10 mg/L
Aniline	Greater than 10 mg/L
Bicarbonate	Greater than 1000 mg/L
Calcium	Greater than 3500 mg/L
Carbonate	Greater than 500 mg/L
Chloride	Greater than 5000 mg/L
Copper	Greater than 1.6 mg/L
Cyclohexylamine	Greater than 250 mg/L
Ethanolamine	Greater than 33 mg/L
Ethylenediamine	Greater than 1.5 mg/L
Glucose	Greater than 1000 mg/L
Glycine	Greater than 1000 mg/L
Iron (Fe^{3+})	Greater than 12 mg/L
Lead	Greater than 100 mg/L
Manganese	Greater than 500 mg/L
Mercury	Greater than 70 mg/L
Morpholine	Greater than 0.36 mg/L
Nitrate	Greater than 1000 mg/L
Nitrite	Greater than 8 mg/L
Phenol	Greater than 1050 mg/L
Phosphate	Greater than 200 mg/L
Silica	Greater than 40 mg/L
Sulfate	Greater than 10,000 mg/L
Urea	Greater than 1000 mg/L
Zinc	Greater than 1000 mg/L

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Formaldehyde Voluette® Ampule Standard, 4000-mg/L CH_2O.
5. Use a TenSette® Pipet to add 0.2 mL of the standard to a 100-mL volumetric Class A flask. Dilute to volume with formaldehyde-free water and mix well. Prepare daily. This is an 8000-µg/L (8-mg/L) formaldehyde standard.
6. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of 8000-µg/L standard, respectively, to each sample and mix thoroughly.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 320-µg/L Formaldehyde Standard Solution by pipetting 1.0 mL of the 8000-µg/L solution from the *Accuracy Check* into a 50-mL mixing cylinder. Dilute to 25.0 mL with formaldehyde-free water. Run the test directly on this sample.

Method Performance

Precision

Standard: 300 µg/L CH_2O

Program	95% Confidence Limits of Distribution
200	285–315 µg/L CH_2O

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	3 µg/L CH_2O

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Formaldehyde reacts with MBTH (3-methyl-2-benzothiazoline hydrazone) and a developing solution to form a blue color in proportion to the formaldehyde concentration. Test results are measured at 630 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Formaldehyde Reagent Set (100 Tests)			22577-00
Includes:			
(1) Developing Solution For Low Range Formaldehyde	5 mL	500 mL	22572-49
(2) MBTH Powder Pillows	2 pillows	100/pkg	22571-69

Required Apparatus

Clippers, for opening powder pillow	1	each	968-00
Cylinder, graduated mixing, 50-mL	2	each	1896-41
Pipet, serological, 5-mL	1	each	532-37
Pipet Filler, safety bulb	1	each	14651-00
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Formaldehyde Standard Solution, 10-mL Voluette® Ampule, 4000-mg/L		16/pkg	22573-10

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Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Method 8030 Calcium and Magnesium; Calmagite Colorimetric Method (0.07 to 4.00 mg/L Ca and Mg as $CaCO_3$)

Scope and Application: For water, wastewater, and seawater

Tips and Techniques

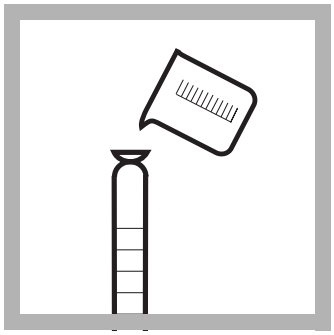
- For the most accurate magnesium test results, keep the sample temperature between 21–29 °C (70–84 °F).
- The test will detect any calcium or magnesium contamination in the mixing cylinder, measuring droppers, or sample cells. To test cleanliness, repeat the test until results are consistent.
- Total hardness in mg/L equals mg/L Ca as $CaCO_3$ plus mg/L Mg as $CaCO_3$.
- Remaining traces of EDTA or EGTA from previous tests will give erroneous results. Rinse sample cells thoroughly before using.

Method 8030

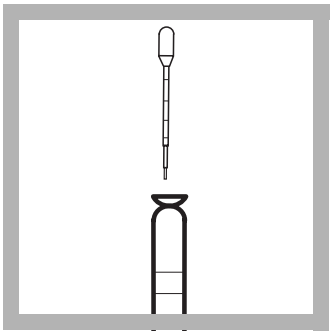
1. Touch **Hach Programs**.

Select program **225 Hardness Mg**.

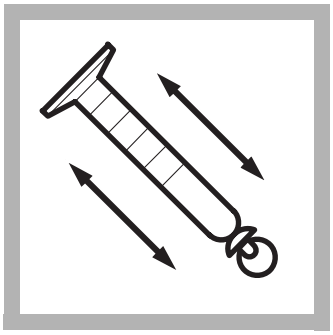
Touch **Start**.



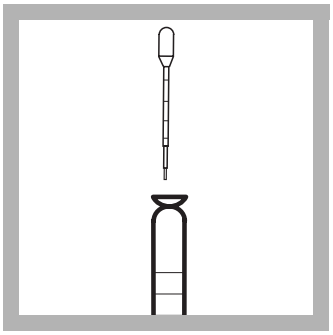
2. Pour 100 mL of sample into a 100-mL graduated mixing cylinder.



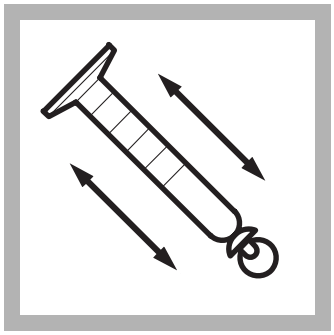
3. Add 1.0 mL of Calcium and Magnesium Indicator solution using a 1.0 mL measuring dropper.



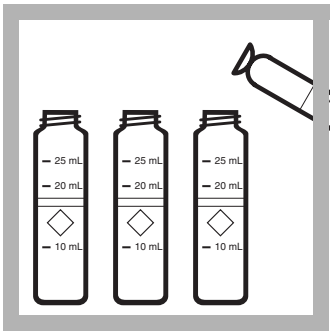
4. Stopper the cylinder and invert it several times.



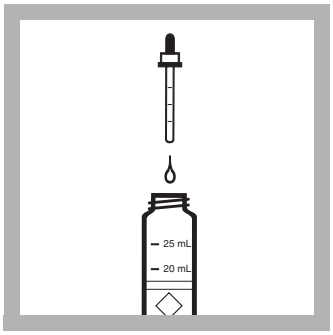
5. Add 1.0 mL of Alkali Solution for Calcium and Magnesium Test using a 1.0 mL measuring dropper.



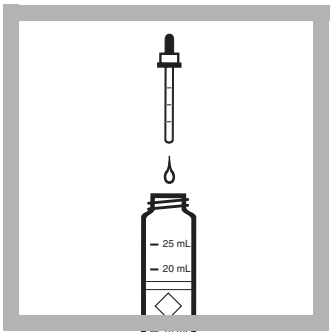
6. Stopper the cylinder and invert it several times.



7. Pour 25 mL of the solution into each of **three**, round sample cells.

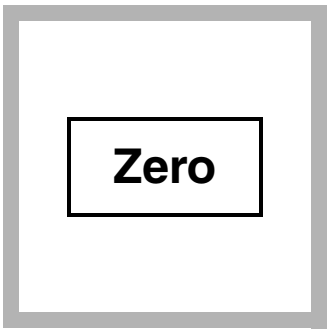


8. Add one drop of 1 M EDTA Solution to the **first** cell (the blank). Swirl to mix.



9. Add one drop of EGTA Solution to the **second** cell. Swirl to mix.

10. Place **first** cell (the blank) into the cell holder.



11. Touch **Zero**.

The display will show:

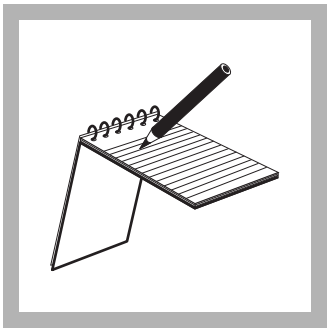
0.00 ppm Mg $CaCO_3$

12. Place the **second** cell into the cell holder.

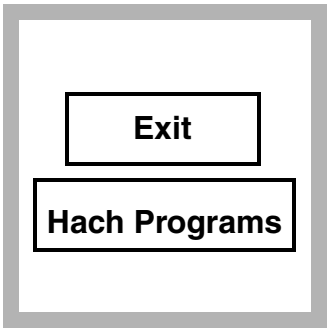
Touch **Read**.

Results will appear in mg/L magnesium as calcium carbonate.

Note: *This value is the amount of* ***magnesium*** *in the sample expressed as $CaCO_3$.*



13. Do not remove the cell from the instrument. Record or store the results.



14. Touch **Exit.**

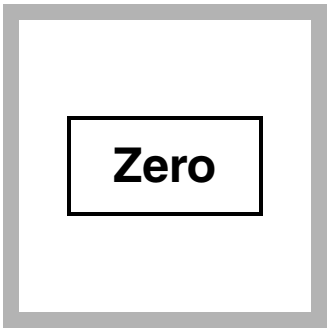
Note: *At this point, sample data may be stored for later access if desired.*

Touch **Hach Programs**.

Select program

220 Hardness, Ca.

Touch **Start**.



15. Touch **Zero**.

The display will show:

0.00 ppm Ca $CaCO_3$

16. Place the **third** cell into the cell holder.

Touch **Read**.

Results will appear in mg/L calcium as calcium carbonate.

Note: *This value is the amount of* ***calcium*** *in the sample expressed as $CaCO_3$.*

Interferences

Interfering Substance	Interference Levels and Treatments
Chromium ($^{3+}$)	Above 0.25 mg/L
Copper ($^{2+}$)	Above 0.75 mg/L
EDTA, chelated	Above 0.2 mg/L as $CaCO_3$
EDTA or EGTA	Traces remaining in sample cells from previous tests will give erroneous results. Rinse cells thoroughly before using.
Iron ($^{2+}$)	Above 1.4 mg/L
Iron ($^{3+}$)	Above 2.0 mg/L
Manganese ($^{2+}$)	Above 0.20 mg/L
Zinc ($^{2+}$)	Above 0.050 mg/L
Ca >1.0 mg/L; Mg >0.25 mg/L	For the most accurate calcium test result, rerun the test on a diluted sample if the calcium is over 1.0 and the magnesium is over 0.25 mg/L as $CaCO_3$. No retesting is needed if either is below those respective concentrations.

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with Nitric Acid (about 5 mL per liter) (Cat. No. 152-49). Cool samples to 4 °C. Preserved samples can be stored up to six months. Before analysis, adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-32). Correct the test results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Method Performance

Precision

Program	Standard as $CaCO_3$	95% Confidence Limits of Distribution as $CaCO_3$
220	2.50 mg/L Ca	2.36–2.64 mg/L Ca
225	2.16 mg/L Mg	2.08–2.24 mg/L Mg

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration as $CaCO_3$
220	Entire range	0.010	0.05 mg/L Ca
225	Entire range	0.010	0.02 mg/L Mg

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The colorimetric method for measuring hardness supplements the conventional titrimetric method because the colorimetric method can measure very low levels of calcium and magnesium. Also, some metals (those listed the table above) that interfere in the titrimetric method may be inconsequential when diluting the sample to bring it within the range of this test. The indicator dye is calmagite, which forms a purplish-blue color in a strongly alkaline solution and changes to red when it reacts with free calcium or magnesium. Calcium and magnesium determinations are made by chelating calcium with EGTA to destroy any red color due to calcium and then chelating the calcium and magnesium with EDTA to destroy the red color due to both calcium and magnesium. By measuring the red color in the different states, calcium and magnesium concentrations are determined. Test results are measured at 522 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Hardness Reagent Set (100 Tests)			23199-00
Includes:			
Alkali Solution for Calcium and Magnesium Test	1 mL	100 mL MDB	22417-32
Calcium and Magnesium Indicator Solution	1 mL	100 mL MDB	22418-32
EDTA Solution, 1 M	1 drop	50 mL SCDB	22419-26
EGTA Solution	1 drop	50 mL SCDB	22297-26

Required Apparatus

Cylinder, 100-mL, graduated mixing	1	each	1896-42
Dropper, measuring, 0.5 and 1.0 mL	2	20/pkg	21247-20
Sample Cells, 10-20-25 mL, w/cap	3	6/pkg	24019-06

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WORLD HEADQUARTERS
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FAX: (970) 669-2932

Method 8374 **Calcium and Magnesium; Chlorophosphonazo Method**

Multi-pathlength Cell **ULR (1 to 1,000 µg/L Ca & Mg as $CaCO_3$)**

Scope and Application: For boiler, cooling, and ultra pure water

Tips and Techniques

- Pre-clean the Pour-Thru Cell and all labware as specified in *Treating Analysis Labware on page 3*.
- Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
- If the sample concentration is greater than 750 µg/L, a 1:1 dilution of the sample is recommended for greatest accuracy. Use ultra-pure (aldehyde-free) water for the dilution. Repeat the analysis on the diluted sample and multiply the resulting concentration by two. See *Correcting for a Diluted Sample* in the instrument manual for more information.
- Alternate forms should only be used when the sample is known to contain only Mg or Ca. This method does not distinguish between the two forms.
- Use dedicated plasticware for this analysis. Plastic sample cells must be used. Glass will contaminate the sample.

Multi-pathlength Cell Method 8374

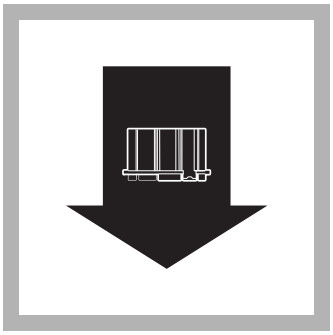
1. Touch

Hach Programs.

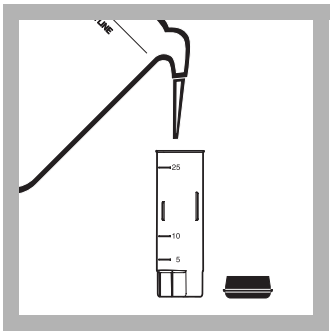
Select program

227 Hardness, Tot. ULR.

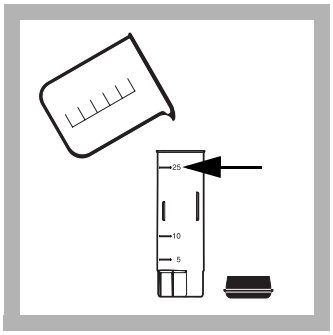
Touch **Start**.



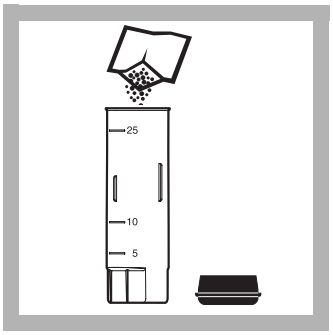
2. Install the cell and cell adapter in the sample cell compartment. Flush with 50-mL of ultra-pure water.



3. Rinse a plastic multi-pathlength sample cell and the cap three times with the water to be tested. Do not allow the underside of the cap to come into contact with surfaces that may contaminate it.



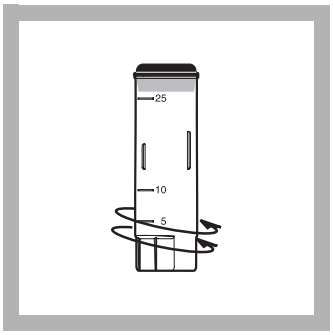
4. Fill the plastic multi-pathlength cell to the 25-mL mark with sample.



5. Add the contents of one Chlorophosphonazo Solution Pillow to the sample cell.

Note: *A small amount of solution may remain in the pillow. This will not affect results.*

Note: *One mL of Chlorophosphonazo Solution (Cat. No. 25895-49) may be used instead of the solution pillow.*

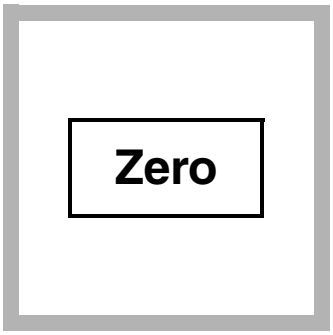


6. Cap the cell and swirl to mix. (This is the blank.)

7. Install the multi-pathlength cell adapter. Use the 25-mm cell pathlength.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

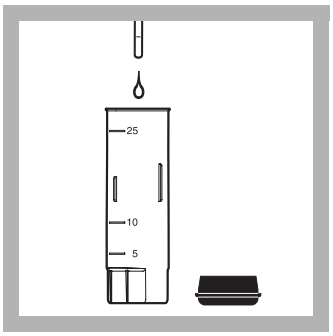
Place the blank into the cell adapter.



8. Touch **Zero**.

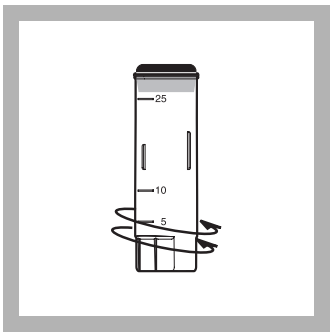
The display will show:

0 µg/L $CaCO_3$



9. Remove the cell from the instrument. Add one drop of CDTA Reagent for Ultra Low Range Hardness.

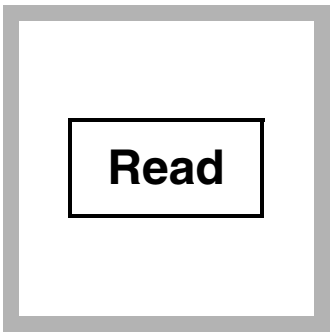
Complete steps 10–11 within 1–2 minutes.



10. Cap the cell and swirl to mix.

11. Place the cell into the multi-pathlength cell adapter.

Use the 25-mm cell pathlength.



12. Touch **Read**.

Results will appear in µg/L $CaCO_3$.

Interferences

Interference studies were conducted at various hardness levels between 0 and 500 µg/L as $CaCO_3$. Various cations and anions were evaluated at levels in the range appropriate to ultra pure water applications. An ion is said to interfere when the resulting concentration is changed by ±10%.

Table 1 Negative Interference

Ion	Level above which the ion interferes (µg/L)
Aluminum	150
Sodium	79,000

Table 2 Positive Interference

Ion	Level above which the ion interferes (µg/L)
Copper	250
Silicon	1000

Table 3 No Interference

Ion	Highest Concentration Tested (µg/L)
Potassium	1000
Ammonium	1000
Formaldehyde	47,000

Treating Analysis Labware

Thoroughly clean all containers used in this test to remove any traces of calcium or magnesium. If possible, use plastic containers for all analysis and storage. Clean containers by normal means, then rinse with ultra-pure (aldehyde-free) water. Fill and soak for 10 minutes with a 1:25 dilution of Chlorophosphonazo Reagent in ultra-pure water. Rinse well with ultra-pure water. Keep containers tightly closed and dedicate them for ULR Hardness only. If containers are rinsed and capped after each use, only occasional soaking is necessary.

Sampling and Storage

Do not use glass containers. Collect samples in clean plastic containers, preferably with screw-type closures. Rinse containers several times with the water to be analyzed before capturing the final sample. Seal to avoid contamination during transport. Analyze as soon as possible.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
2. Touch **Edit.** Change the sample volume to read 25 mL. Change the Standard Addition increments to 0.1 mL, 0.2 mL, and 0.3 mL. Touch **OK** to accept these values. After the values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
3. Obtain a Calcium Chloride Standard Solution, 20-mg/L (20,000-µg/L) as $CaCO_3$.
4. Prepare three sample spikes. Use a TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL, of a 20-mg/L as $CaCO_3$ Calcium Chloride Standard to three 25-mL samples, respectively.
5. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery. Each 0.1 mL addition of standard should cause an increase of 80 µg/L hardness as $CaCO_3$.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* for more information.

Standard Solution Method

Using the 0.50-mg/L (500-µg/L as $CaCO_3$) Calcium Chloride Standard Solution, perform the procedure using the standard in place of the sample.

1. To adjust the calibration curve using the reading obtained with the 500-µg/L as $CaCO_3$ Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternative concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.3 Adjusting the Standard Curve* for more information.

Summary of Method

Calcium and magnesium combine equivalently with the Chlorophosphonazo Indicator to form a colored complex which absorbs light very strongly at 669 nm. One drop of the CDTA reagent breaks up this complex, and the resultant decrease in color is proportional to the amount of calcium and magnesium (as $CaCO_3$) in the sample.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Chlorophosphonazo Indicator Solution Pillows	1	100/pkg	25895-99
CDTA Reagent for Ultra Low Range Hardness	1 drop	10 mL SCDB	25896-36

Required Apparatus

Clippers (Shears) for opening solution pillows	1	each	23694-00
Multi-pathlength Cell Adapter	1	each	59466-00
Sample Cell, Multi-pathlength w/cap	1	6/pkg	59405-06

Required Standards

Calcium Standard Solution, 20-mg/L as $CaCO_3$	946 mL	21246-16
Calcium Standard Solution, 0.50-mg/L as $CaCO_3$	946 mL	20580-16
Water, Ultra-pure (aldehyde-free)	500 mL	25946-49

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

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Method 8374 **Calcium and Magnesium; Chlorophosphonazo Rapid Liquid Method**

Pour-Thru Cell **ULR (1 to 1,000 µg/L Ca & Mg as $CaCO_3$)**

Scope and Application: For boiler, cooling, and ultra pure water

Tips and Techniques

- Pre-clean the Pour-Thru Cell and all labware as specified in *Treating Analysis Labware on page 4*.
- Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
- If the sample concentration is greater than 750 µg/L, a 1:1 dilution of the sample is recommended for greatest accuracy. Use ultra-pure (aldehyde-free) water for the dilution. Repeat the analysis on the diluted sample and multiply the resulting concentration by two. See *Correcting for a Diluted Sample* in the instrument manual for more information.
- Alternate forms should only be used when the sample is known to contain only Mg or Ca. This method does not distinguish between the two forms.
- Use dedicated plastic ware for this analysis.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

DR/2400 Pour-Thru Cell Method 8374

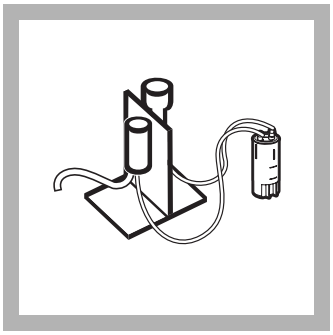
1. Touch

Hach Programs.

Select program

227 Hardness, Tot. ULR.

Touch **Start.**



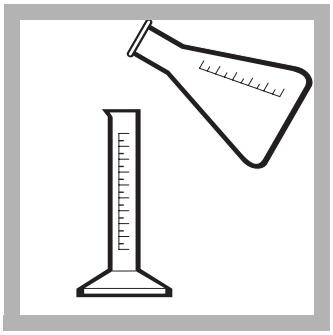
2. Install the Pour-Thru Cell and multi-pathlength cell adapter in the sample cell compartment. Use the 25-mm cell pathlength.

Note: *See Section 2.6.1 on page 26 in the Instrument manual for installation details.*

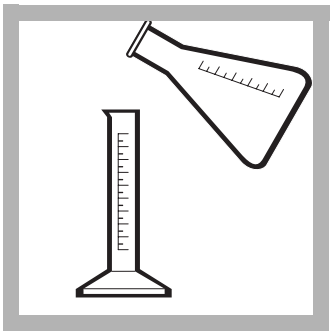
Flush with 50-mL of deionized water.

3. Fill a clean, 125-mL plastic Erlenmeyer flask to overflowing with sample.

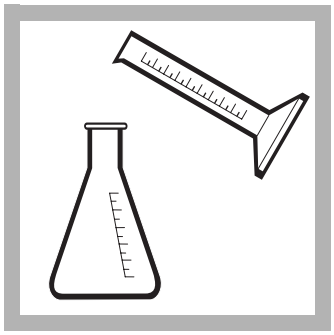
Note: *Collect sample directly in the flask if possible.*



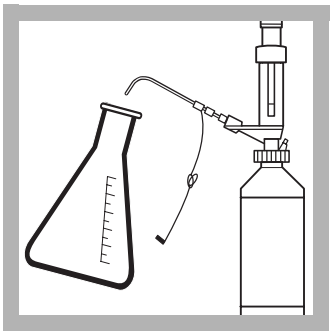
4. Rinse a clean, 50-mL plastic graduated cylinder three times with sample.



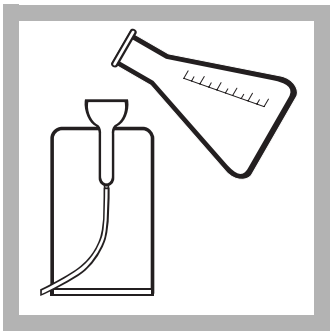
5. Fill this rinsed cylinder to the 50-mL mark with sample from the flask. Discard remaining contents of the flask.



6. Pour the contents of the 50-mL cylinder back into the flask.

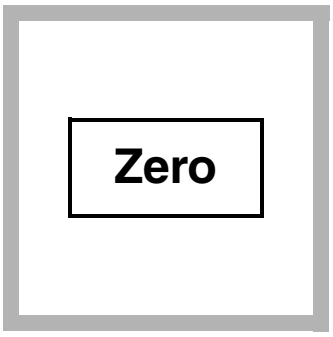


7. Add 2.0 mL of Chlorophosphonazo Reagent to the sample with the Repipet Jr. Dispenser. Swirl to mix.



8. Pour approximately half (25 mL) of the sample into the Pour-Thru Cell.

You may use a clean, dry, plastic 25-mL graduated cylinder to measure the sample.



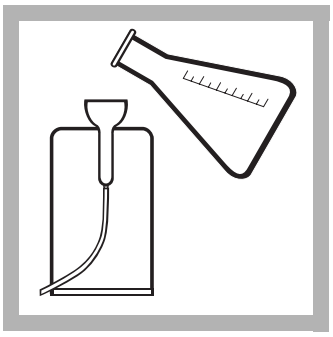
9. After the flow stops, touch **Zero**.

The display will show:

0 µg/L $CaCO_3$

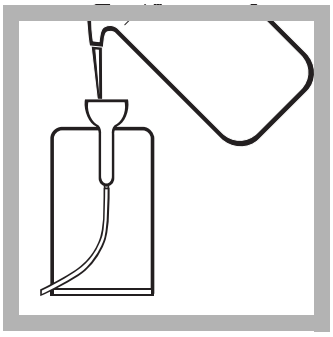
10. Add one drop of CDTA Reagent for Ultra Low Range Hardness to the remaining sample in the flask. Swirl to mix.

Complete steps 11 and 12 within one to two minutes.



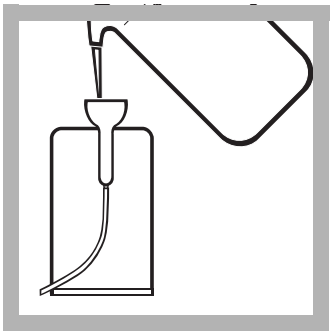
11. Pour the remaining sample into the Pour-Thru Cell.

After the flow stops, touch **Read**. Results will appear in µg/L $CaCO_3$.



12. Using a wash bottle, rinse the Pour-Thru Cell with ultra-pure water immediately after use.

Rinse the flask with ultra-pure water. Cap when finished.



13. Flush the Pour-Thru cell with an additional 50-mL of ultra-pure water.

Interferences

Interference studies were conducted at various hardness levels between 0 and 500 µg/L as $CaCO_3$. Various cations and anions were evaluated at levels in the range appropriate to ultra pure water applications. An ion is said to interfere when the resulting concentration is changed by ±10%.

Table 1 Negative Interference

Ion	Level above which the ion interferes (µg/L)
Aluminum	150
Sodium	79,000

Table 2 Positive Interference

Ion	Level above which the ion interferes (µg/L)
Copper	250
Silicon	1000

Table 3 No Interference

Ion	Highest Concentration Tested (µg/L)
Potassium	1000
Ammonium	1000
Formaldehyde	47,000

Treating Analysis Labware

Clean all containers used in this test thoroughly to remove any traces of calcium or magnesium. If possible, use plastic containers for all analysis and storage. Clean containers by normal means, then rinse with ultra-pure (aldehyde-free) water. Fill and soak for 10 minutes with a 1:25 dilution of Chlorophosphonazo Reagent in ultra-pure water. Rinse well with ultra-pure water. Keep containers tightly closed and dedicate them for ULR Hardness only. If containers are rinsed and capped after each use, only occasional soaking is necessary. Fill the Pour-Thru cell with this same mixture of chlorophosphonazo and water and let stand for several minutes. Rinse with ultra-pure water.

Avoid contamination of the Chlorophosphonazo Reagent bottle when placing the Repipet dispenser on the bottle. Rinse the inlet tubing and inside of the dispenser cap with copious amounts of ultra-pure water using a wash bottle. Place the inlet tubing into a beaker of ultra-pure water and depress the plunger 10–15 times to rinse the inside of the dispenser. (For best results, pour a small amount of reagent into the beaker of rinse water.) Remove the dispenser from the water and depress the plunger until all of the water has been expelled. Shake off any excess water on the dispenser, place the dispenser on the bottle, and tighten.

Sampling and Storage

Do not use glass containers. Collect samples in clean plastic containers, preferably with screw-type closures. Rinse containers several times with the water to be analyzed before capturing the final sample. Seal to avoid contamination during transport. Analyze as soon as possible.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
2. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
3. Obtain a Calcium Chloride Standard Solution, 20-mg/L (20,000-µg/L) as $CaCO_3$.
4. Prepare three sample spikes. Use a TenSette® Pipet to add 0.2 mL, 0.4 mL, and 0.6 mL, of a 20-mg/L as $CaCO_3$ Calcium Chloride Standard to three 50-mL samples, respectively.
5. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery. Each 0.2 mL addition of standard should cause an increase of 80 µg/L hardness as $CaCO_3$.

6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Using the 0.50-mg/L (500-µg/L as $CaCO_3$) Calcium Chloride Standard Solution, perform the procedure using the standard in place of the sample.

1. To adjust the calibration curve using the reading obtained with the 500-µg/L as $CaCO_3$ Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternative concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Summary of Method

Calcium and magnesium combine equivalently with the Chlorophosphonazo Indicator to form a colored complex which absorbs light very strongly at 669 nm. One drop of the CDTA reagent breaks up this complex, and the resultant decrease in color is proportional to the amount of calcium and magnesium (as $CaCO_3$) in the sample.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Chlorophosphonazo Indicator Solution	2 mL	500 mL	25895-49
CDTA Reagent for Ultra Low Range Hardness	1 drop	10 mL SCDB	25896-36

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cylinder, graduated, 50-mL, poly	1	each	1081-41
Dispenser, Fixed-volume, 2.0-mL, Repipet Jr.	1	each	22307-01
Flask, Erlenmeyer, PMP w/cap, 125-mL	1	each	20898-43
Pour-Thru Cell Kit	1	each	59404-00

Required Standards

Calcium Standard Solution, 20-mg/L as $CaCO_3$	946 mL	21246-16
Calcium Standard Solution, 0.50-mg/L as $CaCO_3$	946 mL	20580-16
Water, Ultra-pure (aldehyde-free)	500 mL	25946-49

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Method 8141 **p-Dimethylaminobenzaldehyde Method***

Reagent Solution or AccuVac® Ampuls **(4 to 600 µg/L)**

Scope and Application: For boiler water/feedwater, water, and seawater

* Adapted from *ASTM Manual of Industrial Water*, D1385-78, 376 (1979)

Tips and Techniques

- Samples cannot be preserved and must be analyzed immediately.
- Sample temperature should be 21 ±4 °C (70 ±7 °F).
- After adding the HydraVer® 2 Hydrazine Reagent, a yellow color will develop in the sample if hydrazine is present. The blank may also have a faint yellow color.
- The final samples will have a pH less than 2, which is considered corrosive (0002) by the Federal RCRA.

Reagent Solution Method 8141

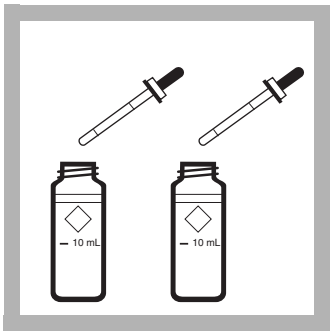
1. Touch **Hach Programs**.

Select program **231 Hydrazine**.

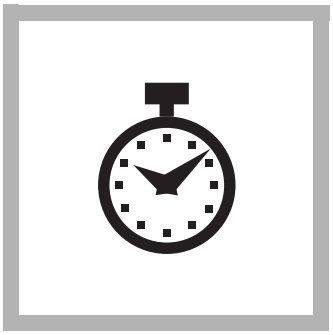
Touch **Start**.

2. Use a graduated cylinder to pour 10 mL of deionized water into a round sample cell (the blank).

3. Use a graduated cylinder to pour 10 mL of sample into a round sample cell (the prepared sample).



4. Add 0.5 mL of HydraVer 2 Hydrazine Reagent to each sample cell. Swirl to mix.

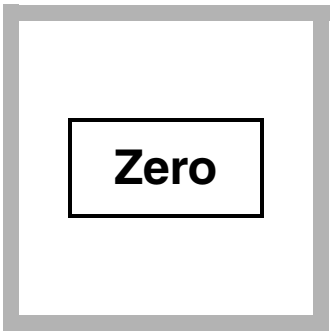


5. Touch the timer icon.

Touch **OK**.

A 12-minute reaction period will begin. Complete steps 6–8 during this period.

6. Place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0 µg/L N_2H_4

8. Place the prepared sample into the cell holder.

Immediately after the timer beeps, touch **Read**. Results will appear in µg/L N_2H_4.

AccuVac Ampul — Method 8141

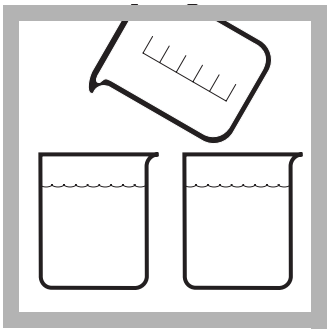
1. Touch

Hach Programs.

Select program

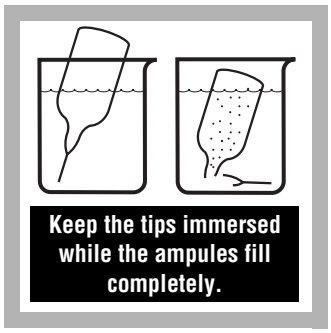
232 Hydrazine AV.

Touch **Start**.



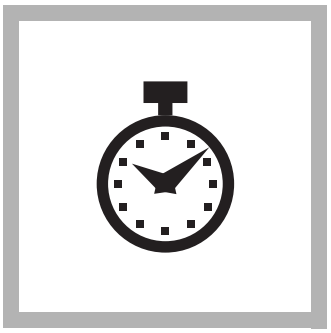
2. Collect at least 40 mL of sample in a 50-mL beaker.

Pour at least 40-mL of deionized water into a second beaker.



3. Fill a HydraVer Hydrazine AccuVac® Ampul with sample.

Fill a second ampule with deionized water (the blank).

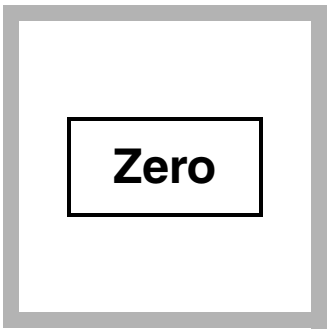


4. Immediately touch the timer icon.

Touch **OK**.

A 12-minute reaction period will begin. Complete steps 5–7 during this period.

5. Place the blank into the cell holder.

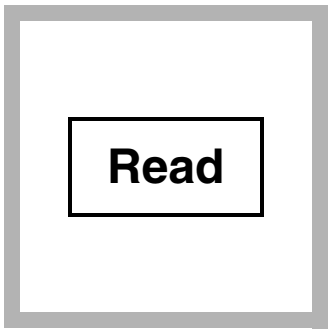


6. Touch **Zero**.

The display will show:

0 µg/L N_2H_4

7. After the timer beeps, place the prepared sample in the cell holder.



8. **Immediately** after the timer beeps, touch **Read**. Results will appear in µg/L N_2H_4.

Interferences

Interfering Substance	Interference Levels and Treatments
Ammonia	No interference up to 10 mg/L. May cause a positive interference of up to 20% at 20 mg/L.
Highly colored or turbid samples	Prepare a blank by oxidizing the hydrazine in a portion of the sample with a 1:1 mixture of deionized water and household bleach. Add one drop of the mixture to 25 mL of sample in a graduated mixing cylinder and invert to mix. Use this solution in step 2, in place of deionized water, to prepare the blank.
Morpholine	No interference up to 10 mg/L.

Sample Collection, Storage and Preservation

Samples collected in glass or plastic bottles should be filled completely and capped tightly. Avoid excessive agitation or exposure to air. Samples must be analyzed immediately after collection and cannot be preserved for later analysis.

Accuracy Check

Standard Solutions Method

1. Prepare a 25-mg/L stock solution. Dissolve 0.1016 g of hydrazine sulfate in 1000 mL of oxygen-free deionized water. Prepare this stock solution daily.
2. Using Class A glassware, prepare a 0.1-mg/L (100-µg/L) hydrazine working solution by diluting 4.00 mL of the 25-mg/L stock solution to 1000 mL with deoxygenated deionized water. Prepare just before analysis. Perform either hydrazine procedure as described above.
3. To adjust the calibration curve using the reading obtained with the 100-µg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected units). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 100 µg/L N_2H_4

Program	95% Confidence Limits of Distribution
231	94–106 µg/L N_2H_4
232	97–103 µg/L N_2H_4

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
231	Entire range	0.010	4 µg/L N_2H_4
232	Entire range	0.010	4 µg/L N_2H_4

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Hydrazine in the sample reacts with the p-dimethylaminobenzaldehyde from the HydraVer 2 Reagent to form a yellow color which is proportional to the hydrazine concentration. Test results are measured at 455 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
HydraVer® 2 Hydrazine Reagent	1 mL	100 mL MDB	1790-32
or Hydrazine Reagent AccuVac® Ampuls	2	25/pkg	25240-25
Water, deionized	10 mL	4 liters	272-56

Required Apparatus (Using Solution)

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Cylinder, graduated, 25-mL	1	each	508-40

Required Apparatus (Using AccuVac® Ampuls)

Beaker, 50-mL	2	each	500-41H

Required Standards

Hydrazine Sulfate, ACS		100 g	742-26

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Method 8031 — DPD Method*

Powder Pillows or AccuVac® Ampuls — (0.07 to 7.00 mg/L)

Scope and Application: For testing dissolved iodine residual used as disinfectant in process water, treated water, estuary water, and seawater

* Adapted from Palin, A.T., *Inst. Water Eng.*, 21 (6), 537-547 (1967).

Tips and Techniques

- Analyze samples immediately. Do not preserve for later analysis.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- If the sample temporarily turns yellow after reagent addition, dilute a fresh sample. Repeat the test. A slight loss of iodine may occur due to the dilution. Apply the appropriate dilution factor. See *Section 2.7 Sample Dilution* on page *21*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows — Method 8031

1. Touch

Hach Programs.

Select program

240 Iodine.

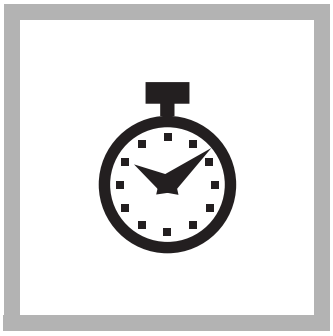
Touch **Start**.

2. Fill a cell with 10 mL of sample.

3. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell (this is the prepared sample).

Swirl to mix.

A pink color will develop if iodine is present.



4. Touch the timer icon.

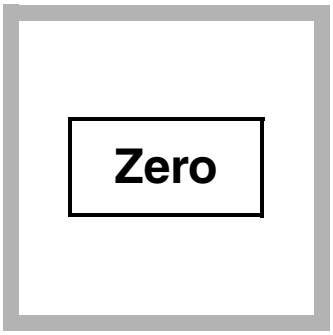
Touch **OK**.

A three-minute reaction period will begin.

5. Fill a second sample cell with 10 mL of sample (this is the blank).

Wipe the blank and place it into the cell holder.

Close the cover.

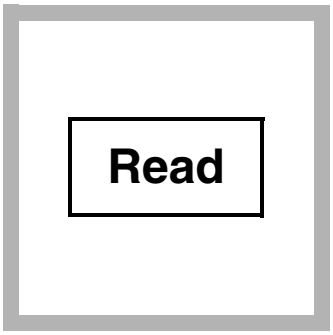


6. Touch **Zero**.

The display will show:

0.00 mg/L I_2

7. Within three minutes after the timer beeps, wipe the prepared sample and place it into the cell holder.



8. Touch **Read**.

Results will appear in mg/L I_2.

AccuVac Ampul — Method 8031

1. Touch

Hach Programs.

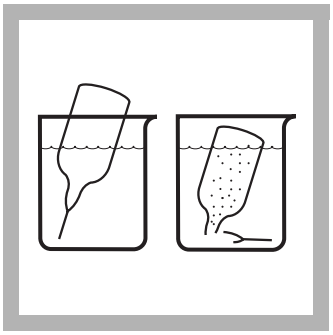
Select program

242 Iodine AV.

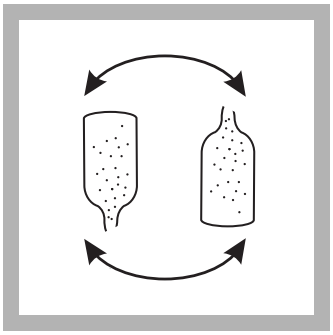
Touch **Start**.

2. Fill a sample cell with 10 mL of sample. (This is the blank.)

Note: *Collect at least 40 mL of sample in a 50-mL beaker.*

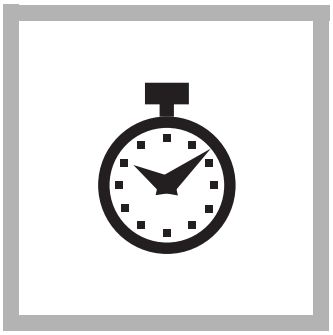


3. Fill a DPD Total Chlorine Reagent AccuVac® Ampul with sample. Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampule several times to mix.

A pink color will develop if iodine is present.

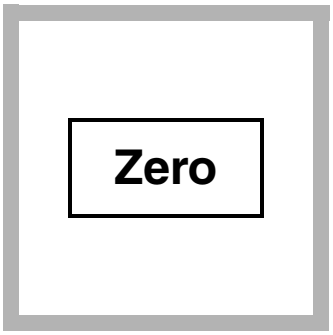


5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin. Perform *steps 6-7* during the reaction period.

6. Wipe the blank and place it into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L I_2

8. Within three minutes after the timer beeps, wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L I_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sodium Hydroxide (Cat. No. 1045-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (See *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Alkalinity	Greater than 250 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N Sulfuric Acid (Cat. No. 1270-32). Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition (See *Section 3.1.3 Correcting for Volume Additions* on page *29*).
Bromine	Interferes at all levels
Chlorine and chloramines	Causes a positive interference at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1000 mg/L as $CaCO_3$
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops Potassium Iodide (30-g/L) (Cat. No. 343-32) to a 25-mL sample. **3.** Mix and wait 1 minute. **4.** Add 3 drops Sodium Arsenite* (5-g/L) (Cat. No. 1047-32) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct iodine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or highly buffered samples	Adjust to pH 6–7. See *Section 3.3 Interferences* on page *41.*

* Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). See *Section 4* for more information on proper disposal of these materials.

Sample Collection, Storage and Preservation

Collect samples in clean, dry glass containers. If sampling from a tap, allow the water to flow at least 5 minutes to ensure a representative sample. Avoid excessive agitation and exposure to sunlight when sampling. Allow several volumes of water to overflow the container and cap the container so there is not headspace above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Proceed with the analysis immediately.

Method Performance

Precision
Standard: 3.83 mg/L I_2

Program	95% Confidence Limits of Distribution
240	3.76–3.90 mg/L I_2
242	3.69–3.97 mg/L I_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
240	Entire range	0.010	0.07 mg/L I_2
242	Entire range	0.010	0.07 mg/L I_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Iodine reacts with DPD (N, N-diethyl-p-phenylenediamine) to form a pink color, the intensity of which is proportional to the total iodine concentration. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
DPD Total Chlorine Reagent Powder Pillows	1 pillow	100/pkg	21056-69
or			
DPD Total Chlorine Reagent AccuVac® Ampuls	1 ampul	25/pkg	25030-25

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

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Method 8147

FerroZine® Reagent Solution

Scope and Application: For water and wastewater

FerroZine® Method*

(0.009 to 1.400 mg/L)

* Adapted from Stookey, L.L., *Anal. Chem.*, 42(7), 779 (1970)

Tips and Techniques

- Digestion is required for total iron determination. See *Section 4* on page *55* for the digestion procedure.
- Rinse glassware with a 1:1 hydrochloric acid solution. Rinse again with deionized water. These two steps will remove iron deposits that can cause slightly high results.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- If preferred, 0.5 mL of FerroZine® Iron Reagent Solution (Cat. No. 2301-53) can be used in place of the solution pillow.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- If the sample contains rust, see *Interferences* on page *2*.
- Use clean clippers, free of rust, and wipe with a dry towel. Do not allow clippers to contact contents of the pillow.
- FerroZine Iron Reagent may crystallize or precipitate when exposed to cold temperatures during shipment. Reagent quality is not affected. Place the reagent in warm water to redissolve.

Method 8147

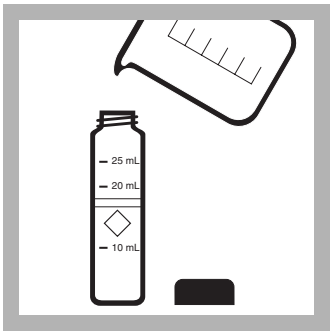
1. Touch

Hach Programs.

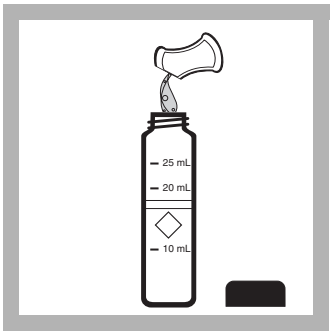
Select program

260 Iron, FerroZine.

Touch **Start**.

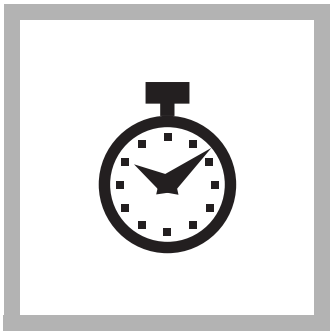


2. Fill a clean, round sample cell to the 25-mL mark with sample.



3. Add the contents of one FerroZine® Iron Reagent Solution Pillow to the sample cell (this is the prepared sample).

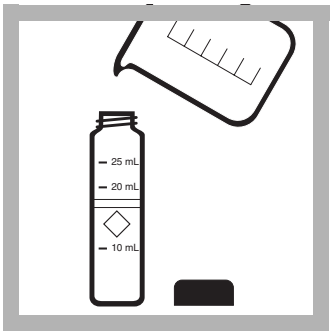
Swirl to mix.



4. Touch the timer icon.

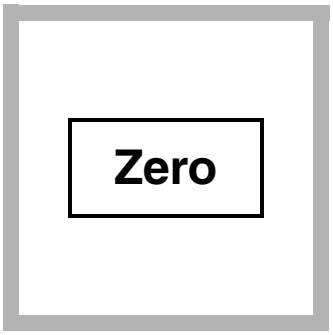
Touch **OK**.

A five-minute reaction period will begin. A violet color will develop if iron is present.



5. Fill another sample cell with 25 mL of sample (this is the blank).

6. When the timer beeps, insert the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.000 mg/L Fe

8. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L Fe.

Interferences

Interfering Substance	Interference Levels and Treatments
Strong chelants (EDTA)	Interfere at all levels. Use the FerroVer® or TPTZ methods for these samples. Use the TPTZ method for low iron concentrations.
Cobalt	May give slightly high results
Copper	May give slightly high results
Hydroxides	Boil the sample, with the FerroZine® Iron Reagent added to it from step 3, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with step 4. Return the sample volume to 25 mL with deionized water. Or, use any of the digestions in *Section 4.*
Magnetite (black iron oxide) or Ferrites	**1.** Fill a 25-mL graduated cylinder with 25 mL of sample. **2.** Transfer this sample into a 125-mL Erlenmeyer flask. **3.** Add the contents of one FerroZine® Iron Reagent Solution Pillow and swirl to mix. **4.** Place the flask on a hot plate or over a flame and bring to a boil. **5.** Continue boiling gently for 20 to 30 minutes. ***Note:*** *Do not allow to boil dry.* ***Note:*** *A purple color will develop if iron is present.* **6.** Return the boiled sample to the 25-mL graduated cylinder. Rinse the Erlenmeyer flask with small amounts of deionized water and empty into the graduated cylinder. **7.** Return the sample volume to the 25-mL mark with deionized water. **8.** Pour this solution into a sample cell and swirl to mix. Proceed with *steps 5–9*. Or, use any of the digestions in *Section 4.*
Rust	Boil the sample, with the FerroZine® Iron Reagent from step 3, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with step 4. Return the sample volume to 25 mL with deionized water. Or, use any of the digestions in *Section 4.*

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with concentrated Nitric Acid, ACS (Cat. No. 152-49) (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature. If you are only reporting dissolved iron, filter the sample immediately after collection and before adding nitric acid.

Before testing, adjust the sample pH to 3–5 with Ammonium Hydroxide, ACS (Cat. No. 106-49). Do not exceed pH 5, or iron may precipitate. Correct test results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off an Iron Voluette® Ampule Standard, 25-mg/L Fe.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Touch the timer icon. After the timer beeps, read the result.
6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Touch the timer icon. After the timer beeps, read the result.
7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Touch the timer icon. After the timer beeps, read the result. Each addition should reflect approximately 100% recovery. Each 0.1 mL of standard should cause a 0.1 mL increase in the concentration reading.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Using Class A glassware, prepare a 1.0-mg/L Fe standard solution by pipetting 5.00 mL of Iron Standard Solution, 100-mg/L, into a 500-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the iron procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 1.0 mg/L Fe

Program	95% Confidence Limits of Distribution
260	0.990–1.010 mg/L Fe

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.009 mg/L Fe

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The FerroZine® Iron Reagent forms a purple-colored complex with trace amounts of iron in samples that are buffered to a pH of 3.5. This method is applicable for determining trace levels of iron in chemical reagents and glycols and can be used to analyze samples containing magnetite (black iron oxide) or ferrites. Test results are measured at 562 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
FerroZine® Iron Reagent Solution	0.5	1000 mL	2301-53
or			
FerroZine® Iron Reagent Solution Pillows	1 pillow	50/pkg	2301-66

Required Apparatus

Clippers, for opening powder pillows	1	each	968-00
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Optional Reagents and Standards

Iron Standard Solution, 100-mg/L Fe		100 mL	14175-42
Iron Standard Solution, 10-mL Voluette® Ampule, 25-mg/L Fe		16/pkg	14253-10
Water, deionized		4 liters	272-56

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1,10-Phenanthroline Method

UniCell™ Vials (0.10 to 5.00 mg/L)

Scope and Application: For water, wastewater, raw water, swimming pool water, and process control; Metal Prep Set digestion is required for determining total iron

Tips and Techniques

- Undissolved iron and complexed iron can only be determined after digestion using the Metal Prep Set (HCT 200). Total iron measuring range is 0.12–6.00 mg/L.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

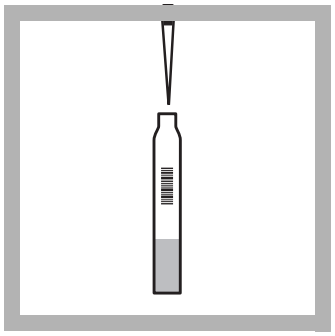
1. Touch

Hach Programs.

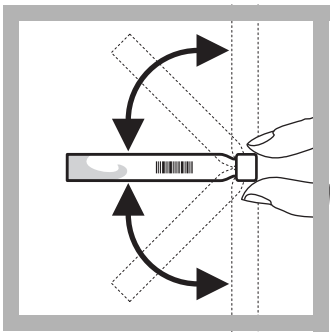
Select program

276 Iron, HCT 159

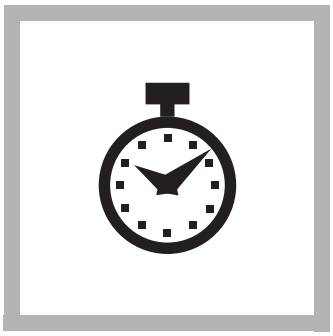
Touch **Start**.



2. Pipet 4 mL of sample into a sample vial.



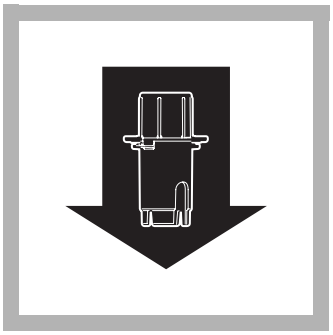
3. Cap and invert the sample until the solid in the vial is completely dissolved.



4. Touch the timer icon.

Touch **OK**.

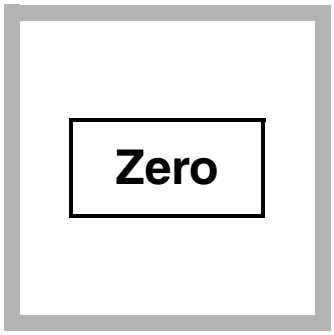
A 15-minute reaction period will begin.



5. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

When the timer beeps, wipe the zero vial (**white** cap) and place it into the cell adapter.



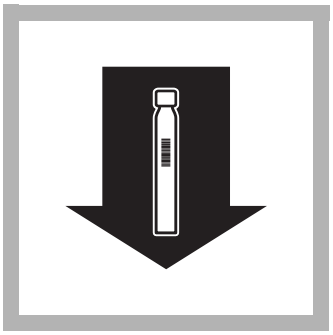
6. Touch **Zero**.

The display will show:

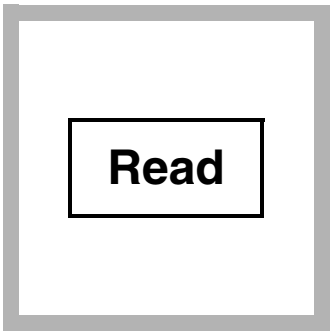
0.00 mg/L Free Fe

Underrange

Note: *If the sample was pre-treated with the* Metal Prep Set (HCT 200)*, touch* **Options** *and select* **Tot. Fe**.



7. Wipe the sample vial and place the prepared sample into the cell holder.



8. Touch **Read**.

Results will appear in mg/L Free Fe.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Cl^-	1000 mg/L
Ca^{2+}	500 mg/L
Ag^+	100 mg/L
Cd^{2+}	70 mg/L
Co^{2+}, Zn^{2+}, Pb^{2+}, CO_3^{2-}, Hg^{2+}, Cr^{3+}, Cr^{6+}	50 mg/L
Ni^{2+}	25 mg/L
Cu^{2+}	10 mg/L
Sn^{2+}	5 mg/L

Higher amounts of copper, nickel, and tin cause a positive interference.

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature. If reporting only dissolved free iron, filter sample immediately after collection and before adding nitric acid.

Before analysis, adjust the pH to between 3 and 5 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 5 or iron may precipitate. Correct the test results for volume additions.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three sample spikes by filling three mixing cylinders with 100 mL of sample. Pipet 0.1 mL, 0.2 mL, and 0.3 mL of 1000-mg/L Fe standard, respectively, to each sample and mix thoroughly.
5. Transfer 4 mL of each solution into a sample vial and analyze as described in the procedure. Touch **Read** to accept each standard additions reading. Each addition should reflect approximately 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 3.00-mg/L Fe standard solution by pipetting 0.3 mL of Iron Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the iron procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 3.00-mg/L Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 3.00 mg/L Free Fe

Program	95% Confidence Limits of Distribution
276	2.63–3.37 mg/L Free Fe

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
276	Entire range	0.010	0.022 mg/L Fee Fe

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Iron (II) ions form an orange-red complex with 1,10-phenanthroline. Any iron (III) ions present in the water sample are reduced to iron (II) ions by ascorbic acid before the complex is formed. Measurements are taken at 485 nm.

Required Reagents

Description	Unit	Cat. No.
Iron - Fe, UniCell™ HCT 159	23/pkg	HCT 159

Required Apparatus

Adapter, 16-mm Cell	each	59457-00
Flask, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 100-mL	each	20886-42
pH Paper	pkg/100	26013-00
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pkg/100	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	pkg/400	27950-00

Optional Reagents

Description	Unit	Cat. No.
Iron Standard 1000-mg/L as Fe	100 mL	2271-42
Metal-Prep-Set HCT 200	50 digestions	HCT 200
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide, 5 N	1L	2450-53

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 8147 **FerroZine® Rapid Liquid Method***

Pour-Thru Cell **(0.009 to 1.400 mg/L Fe)**

Scope and Application: For boiler, cooling, and natural waters*

* Adapted from Stookey, L.L., *Anal.Chem.,* 42 (7) 779 1970.

Tips and Techniques

- If sample contains rust, see *Interferences on page 2*.
- Digestion is required for total iron determination.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- If iron is present, a purple color will form after adding the reagent.
- Rinse glassware with a 1:1 HCl (Cat. No. 884-49) solution. Rinse again with deionized water. This will remove residual iron that may interfere.
- FerroZine Iron Reagent may crystallize or precipitate when exposed to cold temperatures during shipment; reagent quality is not affected. Place the reagent in warm water to re-dissolve.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

Pour-Thru Cell Method 8147

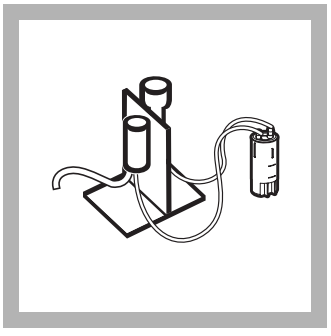
1. Touch

Hach Programs.

Select program

260 Iron, FerroZine RL.

Touch **Start**.



2. Install the Pour-Thru Cell and cell adapter in the sample cell compartment. Use the 25-mm cell pathlength.

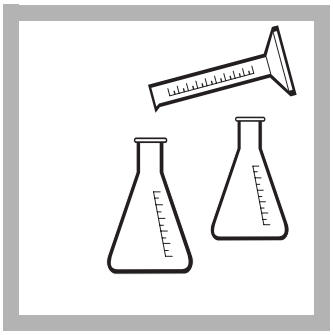
Note: *See Section 2.6.1 on page 26 in the Instrument manual for installation details.*

Flush with 50-mL of deionized water.

3. Rinse two clean 125-mL Erlenmeyer flasks with the sample three times.

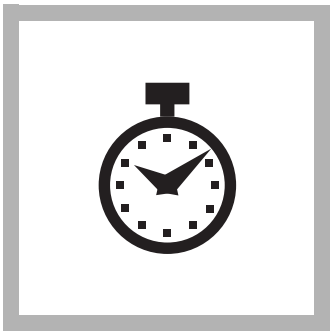
4. Rinse a clean 50-mL plastic graduated cylinder three times with the sample.

5. Fill the rinsed cylinder to the 50-mL mark with sample.



6. Pour the contents of the 50-mL cylinder into one of the flasks. Measure a second 50 mL portion of sample into the graduated cylinder and pour the contents into the second flask.

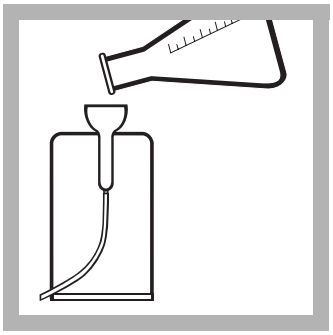
7. Add 1.0 mL of FerroZine Iron Reagent Solution to one of the flasks using the Repipet Dispenser. Swirl to mix. The remaining flask will be used as the blank.



8. Touch the timer icon.

Touch **OK**.

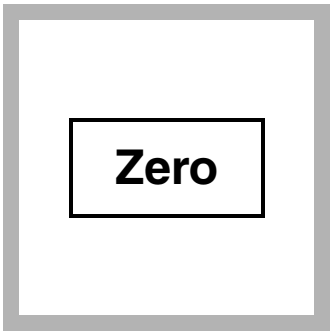
A five-minute reaction period will begin.



9. When the timer beeps, the display will show:

mg/L Fe

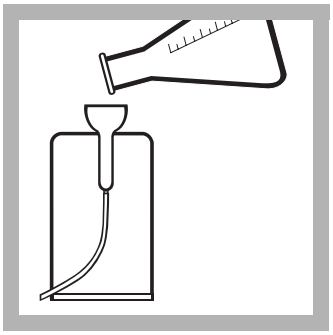
Pour the contents of the flask containing the blank into the Pour-Thru Cell.



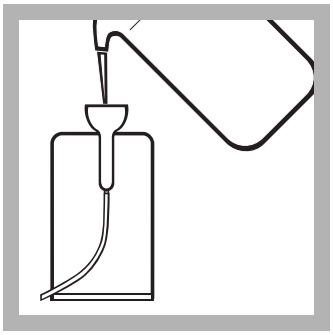
10. When the flow stops, touch **Zero**.

The display will show:

0.000 mg/L Fe



11. Pour the contents of the flask containing the prepared sample into the Pour-Thru Cell. After the flow stops, touch **Read**. Results will appear in mg/L Fe.



12. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Interfering Substance	Interference Levels
Strong chelants (EDTA)	Interfere at all levels. Use the FerroVer® or TPTZ methods for these samples. Use the TPTZ method for low iron concentrations.
Cobalt	May give slightly high results
Copper	May give slightly high results
Hydroxides	Boil the sample, with the FerroZine® Iron Reagent added to it from *step 7*, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with *step 8*. Return the sample volume to 25 mL with deionized water.

(continued)

Interfering Substance	Interference Levels
Magnetite (black iron oxide) or Ferrites	**1.** Fill a 50-mL graduated cylinder with 50 mL of sample. **2.** Transfer the sample into a clean glass 125-mL Erlenmeyer flask. **3.** Add 1.0-mL of FerroZine Iron Reagent Solution Cat. No. 2301-53) and swirl to mix. **4.** Place the flask on a hot plate or over a flame and bring to a boil. **5.** Continue boiling gently for 20 to 30 minutes. ***Note:*** *Do not allow to boil dry.* **6.** Return the boiled sample to the graduated cylinder. Rinse the Erlenmeyer flask with small amounts of deionized water and empty into the graduated cylinder. ***Note:*** *A purple color will develop if iron is present.* **7.** Return the sample volume to the 50-mL mark with deionized water. **8.** Pour the solution into a 125-mL Erlenmeyer flask and swirl to mix. Proceed with *steps 5–9*.
Rust	Boil the sample, with the FerroZine® Iron Reagent from *step 7*, for 1 minute in a boiling water bath. Cool to 24 °C (75 °F) before proceeding with *step 8*. Return the sample volume to 25 mL with deionized water.

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with Nitric Acid (Cat. No. 152-49) (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature. Before testing, adjust the pH of the sample to 3–5 with NaOH ACS (Cat. No. 2450-26); see *3.1.3 Correcting for Volume Additions*

Note: *Do not exceed pH 5 as iron may precipitate.*

If only dissolved iron is to be reported, filter sample immediately after collection and before addition of nitric acid.

Labware

All containers used in this test must be cleaned thoroughly to remove any traces of iron. Rinse labware and the Pour-Thru Cell with a 1:1 HCl solution (Cat. No. 884-49) or with a 1:25 dilution of FerroZine® Reagent. Rinse several times with deionized water.

Keep flasks tightly closed when not in use. Dedicate these containers for iron analysis only. If containers are rinsed and capped after each use, only occasional treatment with HCl or FerroZine® is necessary.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. Remove the color by rinsing with a 1:5 dilution of Ammonium Hydroxide (Cat. No. 106-49), followed by several rinses with deionized water. Cover the Pour-Thru Cell when it is not in use.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Snap the neck off an Iron Voluette® Ampule Standard, 25-mg/L Fe.

2. Use the TenSette® pipet to add 0.2, 0.4, and 0.6 mL of standard to three 50-mL samples, respectively.
3. Analyze each sample as described above. The iron concentration should increase 0.1 mg/L for each 0.2 mL of 25-mg/L standard added.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

Prepare a 1.0 mg/L iron working solution as follows:

1. Pipet 5.00 mL of iron standard solution, 100-mg/L Fe, into a 500-mL volumetric flask.
2. Dilute to volume with deionized water. Prepare this solution daily. Analyze the working solution according to the above procedure.

Method Performance

Precision

Standard: 1.000 mg/L Fe

Program	95% Confidence Limits of Distribution
260	0.990–1.010 mg/L Fe

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.009 mg/L Fe

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The FerroZine® Iron Reagent forms a purple colored complex with trace amounts of iron in samples that are buffered to a pH of 3.5. This method is applicable for determining trace levels of iron in chemical reagents and glycols and can be used to analyze samples containing magnetite (black iron oxide) or ferrites. The test results are measured at 562 nm.

Required Apparatus

Description	Quantity Required Per Test	Unit	Cat. No.
Cylinder, graduated, 50-mL, poly		each	1081-41
Dispenser, Fixed Volume, 1.0-mL, Repipet Jr.		each	21113-01
Flask, Erlenmeyer, PMP w/cap, 125-mL	2	each	20898-43
Pour-Thru Cell Kit		each	59404-00

Required Standards

FerroZine® Iron Reagent Solution	500 mL	2301-49
Iron Standard Solution, 100-mg/L Fe	100 mL	14175-42
Iron Standard Solution, Voluette® ampule, 25-mg/L Fe, 10-mL	16/pkg	14253-10
Water, deionized	4 L	272-56

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HACH COMPANY
WORLD HEADQUARTERS
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FAX: (970) 669-2932

Iron, Ferrous

Method 8146 — **1, 10 Phenanthroline Method***

Powder Pillows or AccuVac® Ampuls — **(0.02 to 3.00 mg/L)**

Scope and Application: For water, wastewater, and seawater

* Adapted from *Standard Methods for the Examination of Water and Wastewater,* 15th ed. 201 (1980)

Tips and Techniques

- Analyze samples as soon as possible to prevent air oxidation of ferrous iron to ferric iron, which is not determined.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- If ferrous iron is present, an orange color will form after adding the reagent.

Powder Pillows — Method 8146

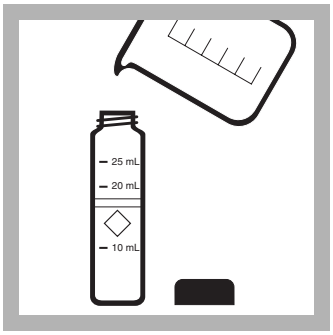
1. Touch

Hach Programs.

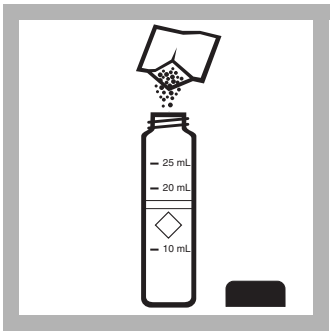
Select program

255 Iron, Ferrous.

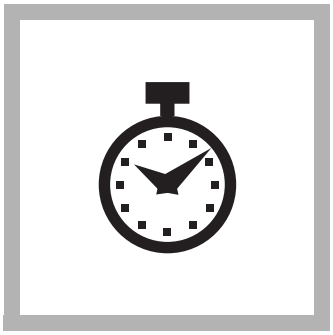
Touch **Start**.



2. Fill a clean, round sample cell with 25 mL of sample.



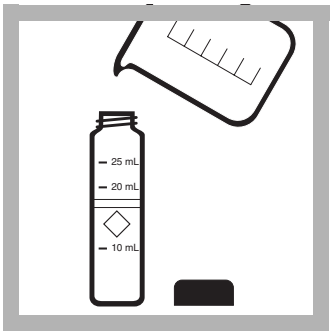
3. Add the contents of one Ferrous Iron Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.



4. Touch the timer icon.

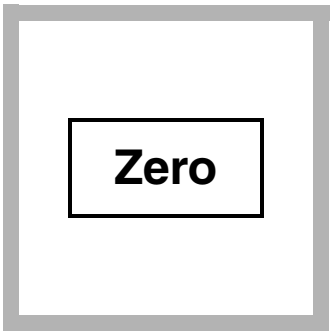
Touch **OK**.

A three-minute reaction period will begin.



5. Fill a second round sample cell with 25 mL of sample (the blank).

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L Fe^{2+}

8. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L Fe^{2+}.

AccuVac Ampul — Method 8146

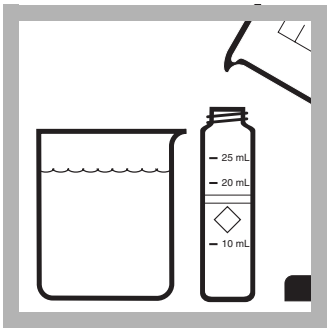
1. Touch

Hach Programs.

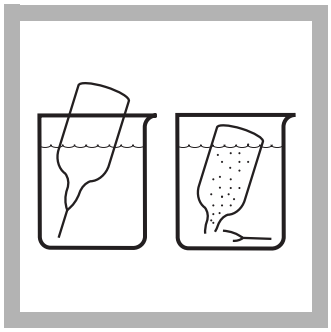
Select program

257 Iron, Ferrous AV.

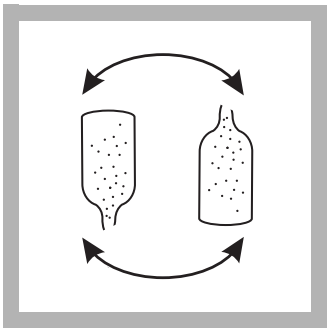
Touch **Start**.



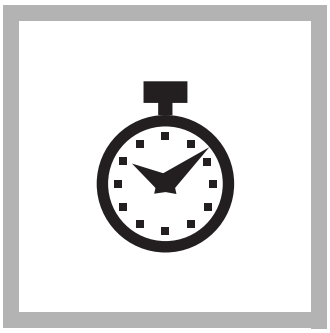
2. Fill a sample cell with 25 mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.



3. Fill a Ferrous Iron AccuVac® Ampul with sample. Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampule several times to mix. Wipe off any liquid or fingerprints.

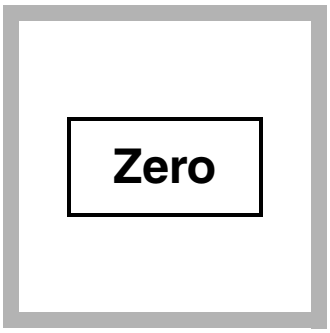


5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L Fe^{2+}

8. Place the AccuVac Ampul into the cell holder.

Touch **Read**.

Results will appear in mg/L Fe^{2+}.

Sample Collection, Storage and Preservation

Collect samples in plastic or glass bottles. Analyze samples as soon as possible after collection.

Accuracy Check

Standard Solution Method

1. Prepare a ferrous iron stock solution (100-mg/L Fe^{2+}) by dissolving 0.7022 grams of Ferrous Ammonium Sulfate, hexahydrate, in deionized water. Dilute to one liter in a Class A volumetric flask. In a 100-mL Class A volumetric flask, dilute 1.00 mL of this solution to 100 mL with deionized water to make a 1.0-mg/L standard solution. Prepare this solution immediately before use. Perform the iron procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L Fe^{2+} Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 1.000 mg/L Fe

Program	95% Confidence Limits of Distribution
255	0.989–1.011 mg/L Fe
257	0.977–1.023 mg/L Fe

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
255	Entire range	0.010	0.028 mg/L Fe
257	Entire range	0.010	0.023 mg/L Fe

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The 1,10 phenanthroline indicator in the Ferrous Iron Reagent reacts with ferrous iron in the sample to form an orange color in proportion to the iron concentration. Ferric iron does not react. The ferric iron (Fe^{3+}) concentration can be determined by subtracting the ferrous iron concentration from the results of a total iron test. Test results are measured at 510 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Ferrous Iron Reagent AccuVac® Ampuls	1 ampul	25/pkg	25140-25
or			
Ferrous Iron Reagent Powder Pillows	1 pillow	100/pkg	1037-69

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Ferrous Ammonium Sulfate, hexahydrate, ACS	113 g	11256-14
Water, deionized	4 liters	272-56

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Iron, Total

★Method 8008

FerroVer® Method*

Powder Pillows or AccuVac® Ampuls

(0.02 to 3.00 mg/L)

Scope and Application: For water, wastewater, and seawater; digestion is required for determining total iron; USEPA approved for reporting wastewater analysis**

* Adapted from *Standard Methods for the Examination of Water and Wastewater*
** *Federal Register*, June 27, 1980; 45 (126:43459)

Tips and Techniques

- Digestion is required for determining total iron for EPA reporting purposes. See the *Hach Water Analysis Handbook* for digestion procedures.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- After adding reagent, an orange color will form if iron is present.
- Accuracy is not affected by undissolved powder.

Powder Pillows — Method 8008

1. Touch

Hach Programs.

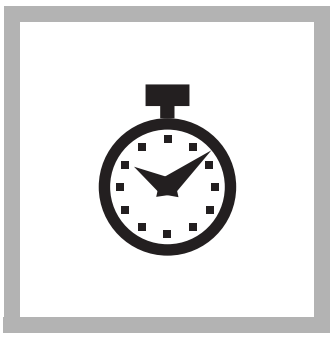
Select program

265 Iron, FerroVer.

Touch **Start**.

2. Fill a clean, round sample cell with 10 mL of sample.

3. Add the contents of one FerroVer Iron Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.



4. Touch the timer icon.

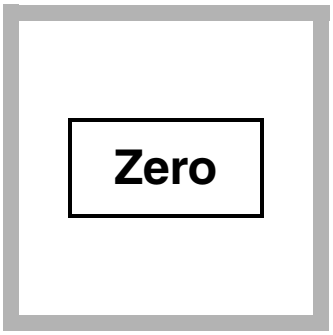
Touch **OK**.

A three-minute reaction period will begin.

(Allow samples that contain rust to react for at least 5 minutes.)

5. Fill another sample cell (the blank) with 10 mL of sample.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L Fe

8. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L Fe.

AccuVac Ampul — Method 8008

1. Touch

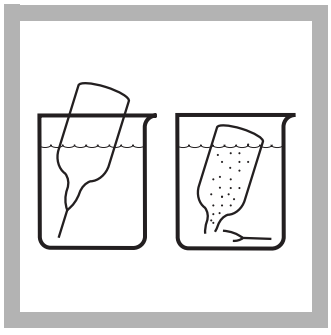
Hach Programs.

Select program

267 Iron, FerroVer AV.

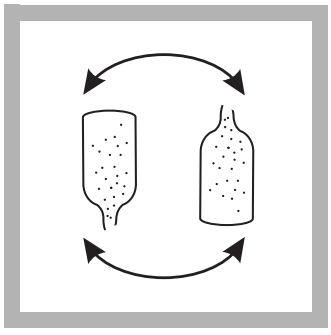
Touch **Start**.

2. Fill a sample cell with 25 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.

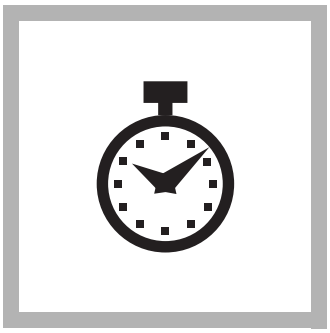


3. Fill a FerroVer Iron AccuVac® Ampul with sample.

Keep the tip immersed while the ampule fills completely.



4. Quickly invert the ampule several times to mix. Wipe off any liquid or fingerprints.



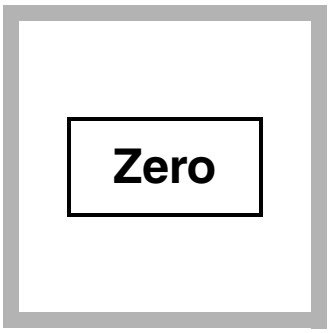
5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.

(Samples that contain rust should react for at least 5 minutes.)

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L Fe

8. Place the AccuVac Ampul into the cell holder.

Touch **Read**.

Results will appear in mg/L Fe.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium, Ca^{2+}	No effect at less than 10,000 mg/L as $CaCO_3$.
Chloride, Cl^-	No effect at less than 185,000 mg/L.
Copper, Cu^{2+}	No effect. Masking agent is contained in FerroVer Reagent.
High Iron Levels	Inhibit color development. Dilute sample and re-test to verify results.
Iron Oxide	Requires mild, vigorous or Digesdahl digestion. After digestion, adjust sample to pH 3–5 with sodium hydroxide (Cat. No. 2450-32), then analyze.
Magnesium	No effect at 100,000 mg/L as calcium carbonate.
Molybdate Molybdenum	No effect at 50 mg/L as Mo.
High Sulfide Levels, S^{2-}	**1.** Treat in fume hood or well-ventilated area. Add 5 mL hydrochloric acid, ACS (Cat. No. 134-49) to 100 mL sample in a 250-mL Erlenmeyer flask. Boil 20 minutes. **2.** Cool. Adjust pH to 3–5 with Sodium Hydroxide (Cat. No. 2450-32). Readjust volume to 100 mL with deionized water. **3.** Analyze.
Turbidity	**1.** Add 0.1 g scoop of RoVer® Rust Remover (Cat. No. 300-01) to the blank. Swirl to mix. **2.** Zero the instrument with this blank. **3.** If sample remains turbid, add three 0.2 g scoops of RoVer to a 75-mL sample. Let stand 5 minutes. **4.** Filter through a Glass Membrane Filter (Cat. No. 2530-00) and Filter Holder (Cat No. 2340-00). **5.** Use filtered sample in steps 2 and 5.
Extreme Sample pH	Adjust pH to 3–5. See *Section 3.3 Interferences* on page *41.*
Highly Buffered Samples	Adjust pH to 3–5. See *Section 3.3 Interferences* on page *41.*

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the sample immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter) (Cat. No. 152-49). Preserved samples may be stored up to six months at room temperature. Before analysis, adjust the pH to between 3 and 5 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-32). Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

If only dissolved iron is to be determined, filter the sample before acid addition.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off an Iron Voluette Ampule Standard, 50-mg/L.
5. Prepare a 0.1 mL sample spike by adding 0.1 mL of standard to the unspiked sample. Touch the timer icon. After the timer beeps, read the result.
6. Prepare a 0.2 mL sample spike by adding 0.1 mL of standard to the 0.1 mL sample spike. Touch the timer icon. After the timer beeps, read the result.
7. Prepare a 0.3 mL sample spike by adding 0.1 mL of standard to the 0.2 mL sample spike. Touch the timer icon. After the timer beeps, read the result. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac Ampuls, fill three mixing cylinders (Cat. No. 1896-41) with 50-mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 1.00-mg/L Fe standard solution by pipetting 1.00 mL of Iron Standard Solution, 100-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the iron procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 1.00 mg/L Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.

3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 1.000 mg/L Fe

Program	95% Confidence Limits of Distribution
265	0.989–1.011 mg/L Fe
267	0.977–1.023 mg/L Fe

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
265	Entire range	0.010	0.022 mg/L Fe
267	Entire range	0.010	0.023 mg/L Fe

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

FerroVer Iron Reagent converts all soluble iron and most insoluble forms of iron in the sample to soluble ferrous iron. The ferrous iron reacts with the 1,10 phenanthroline indicator in the reagent to form an orange color in proportion to the iron concentration. Test results are measured at 510 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
FerroVer® Iron Reagent Powder Pillows (for 10-mL sample)	1 pillow	100/pkg	21057-69
or			
FerroVer® Iron Reagent AccuVac® Ampuls	1 ampul	25/pkg	25070-25

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Beaker, 50-mL	1	each	500-41H

Required Standards

Iron Standard Solution, 100-mg/L		100 mL	14175-42
Iron Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as Fe		16/pkg	14254-10
Water, deionized		4 liters	272-56

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3/04 1ed

Iron, Total

Method 8112

TPTZ Method*

Powder Pillows or AccuVac® Ampuls

(0.012 to 1.800 mg/L)

Scope and Application: For water, wastewater, and seawater

* Adapted from G. Frederic Smith Chemical Co., *The Iron Reagents*, 3rd ed. (1980)

Tips and Techniques

- Digestion is required for determining total iron. See the *Hach Water Analysis Handbook* for digestion procedures.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Rinse all glassware with a 1:1 Hydrochloric Acid Solution (Cat. No. 884-49). Rinse again with deionized water. This process will remove iron deposits that can cause slightly high results.
- Wipe all blanks, sample cells, and ampules with a damp cloth followed by a dry cloth to reduce fingerprints and other marks before placing in the instrument.
- After adding reagent, a blue color will develop if iron is present.
- Adjust the pH of stored samples to 3–4. Do not exceed pH 5 or iron may precipitate.

TPTZ Powder Pillows — Method 8112

1. Touch

Hach Programs.

Select program

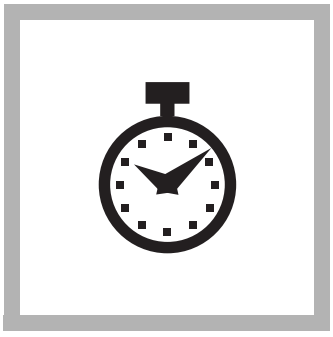
270 Iron, Total TPTZ.

Touch **Start**.

2. Fill one clean, round sample cell with 10 mL of sample (this is the prepared sample).

Fill another cell with 10 mL of deionized water (this is the blank).

3. Add the contents of one 10-mL TPTZ Iron Reagent Powder Pillow to the prepared sample. Stopper and shake for 30 seconds. Remove cap.



4. Touch the timer icon.

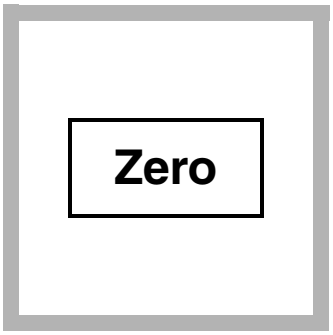
Touch **OK**.

A three-minute reaction period will begin.

Proceed to *step 5* while the timer is running.

5. Add the contents of one 10-mL TPTZ Iron Reagent Powder Pillow to the reagent blank. Cap and shake for 30 seconds.

6. When the timer beeps, insert the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.000 mg/L Fe

8. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L Fe.

TPTZ AccuVac® Ampul — Method 8112

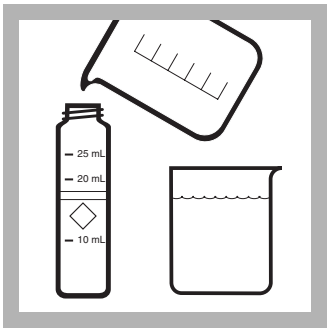
1. Touch

Hach Programs.

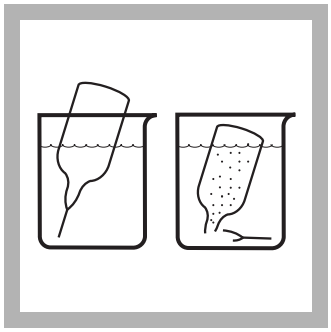
Select program

272 Iron, TPTZ AV.

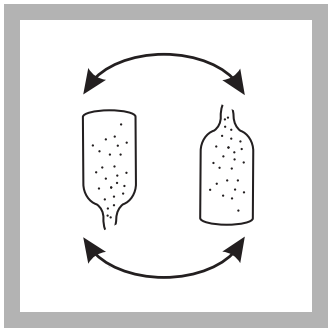
Touch **Start**.



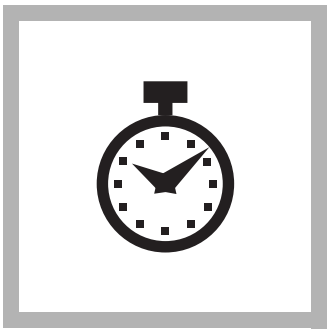
2. Collect at least 40 mL of sample in a 50-mL beaker. Fill a sample cell with 25 mL of sample (this is the blank).



3. Fill a TPTZ Iron AccuVac® Ampul with sample. Keep the tip immersed while the ampule fills completely.



4. Invert the ampule repeatedly to mix.



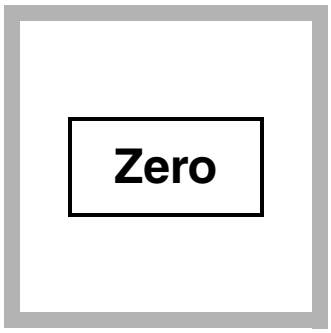
5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.

Complete *step 6* during this period.

6. When the timer beeps, place the blank into the adapter.



7. Touch **Zero**.

The display will show:

0.000 mg/L Fe

8. Place the prepared sample into the adapter.

Touch **Read**.

Results will appear in mg/L Fe.

Interferences

Interference tests were performed using an iron concentration of 0.5 mg/L. When interferences occurred, the color formation was inhibited or a precipitate formed. The following do not interfere with the test when present up to the levels given.

Interfering Substance	Interference Levels and Treatments
Cadmium	4.0 mg/L
Chromium ($^{3+}$)	0.25 mg/L
Chromium ($^{6+}$)	1.2 mg/L
Cobalt	0.05 mg/L
Copper	0.6 mg/L
Cyanide	2.8 mg/
Manganese	50.0 mg/L
Mercury	0.4 mg/L
Molybdenum	4.0 mg/L
Nickel	1.0 mg/L
Nitrite Ion	0.8 mg/L
Color or turbidity	In the powder pillow procedure, if the sample, without a TPTZ Iron Reagent Powder Pillow, has a color or turbidity greater than the blank (deionized water plus TPTZ Iron Reagent), then use the sample as the blank.
pH	A sample pH of less than 3 or greater than 4 after the addition of reagent may inhibit color formation, cause the developed color to fade quickly, or to result in turbidity. Adjust the sample pH in the sample cell before the addition of reagent to between 3 and 4 by using a pH meter or pH paper and adding dropwise an appropriate amount of iron-free acid or base such as 1.0 N Sulfuric Acid Standard Solution (Cat. No. 1270-32) or 1.0 N Sodium Hydroxide Standard Solution (Cat. No. 1045-32). Make a volume correction if significant volumes of acid or base are used; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with about 2 mL/L Nitric Acid, ACS (Cat. No. 152-49). Store preserved samples up to six months at room temperature. If reporting only dissolved iron, filter sample immediately after collection and before adding nitric acid.

Before testing, adjust the pH of the stored sample to between 3–4 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-26). Do not exceed pH 5 as iron may precipitate. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off an Iron Voluette® Ampule Standard, 10-mg/L Fe.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 10 mL of sample. Use the TenSette® Pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of standard, respectively, to each sample and mix thoroughly.

Note: *For AccuVac® Ampuls, fill three mixing cylinders (Cat. No. 1896-41) each with 50-mL of sample and spike with 0.4 mL, 0.8 mL, and 1.2 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Using Class A glassware, prepare a 1.000-mg/L iron standard solution by pipetting 5.00 mL of Iron Standard Solution, 100-mg/L, into a 500-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the iron procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 1.000 mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.

3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 1.000 mg/L Fe

Program	95% Confidence Limits of Distribution
270	0.983–1.017 mg/L Fe
272	0.985–1.015 mg/L Fe

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
270	Entire range	0.010	0.012 mg/L Fe
272	Entire range	0.010	0.012 mg/L Fe

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The TPTZ Iron Reagent forms a deep blue-purple color with ferrous iron. The indicator is combined with a reducing agent which coverts precipitated or suspended iron, such as rust, to the ferrous state. The amount of ferric iron present can be determined as the difference between the results of a ferrous iron test and the concentration of total iron. Test results are measured at 590 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
TPTZ Iron Reagent Powder Pillows (for 10-mL sample)	2 pillows	100/pkg	26087-99
or			
TPTZ Low Range Iron Reagent AccuVac® Ampuls	1 ampule	25/pkg	25100-25

Required Apparatus

Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Beaker, 50-mL	1	each	500-41H

Optional Reagents and Standards

Iron Standard Solution, 100-mg/L Fe		100 mL	14175-42
Iron Standard Solution, 10-mL Voluette® Ampule, 25-mg/L Fe		16/pkg	14253-10
Water, deionized		4 liters	272-56

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3/04 1ed

Iron, Total

Method 8365 — **FerroMo Method***

Powder Pillows — **(0.01 to 1.80 mg/L)**

Scope and Application: For cooling water containing molybdate-based treatment

* Adapted from G. Frederick Smith Chemical Co., *The Iron Reagents*, 3rd ed. (1980)

Tips and Techniques

- Digestion is required for total iron determination. See the *Hach Water Analysis Handbook* for digestion procedures.
- Rinse glassware with a 1:1 hydrochloric acid solution. Rinse again with deionized water. These two steps will remove iron deposits that can cause slightly high results.
- After the addition of the reagent, the sample pH should be between 3–5.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- If the sample contains high levels of molybdate (100 mg/L MoO_4^{2-} or greater), read the sample immediately after zeroing the blank.

Powder Pillows — Method 8365

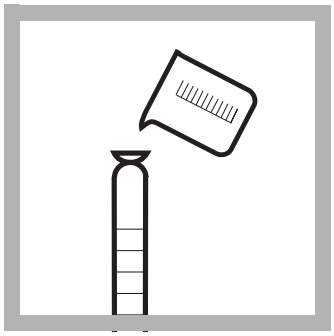
1. Touch

Hach Programs.

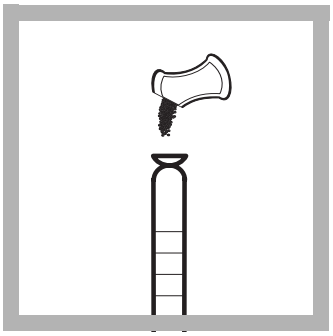
Select program

275 Iron, FerroMo.

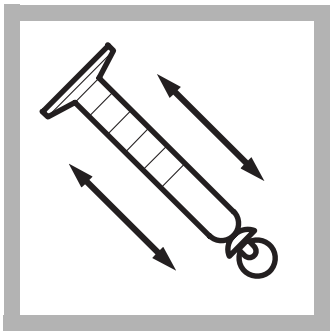
Touch **Start**.



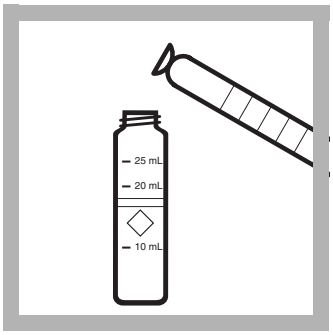
2. Fill a 50-mL graduated mixing cylinder with 50 mL of sample.



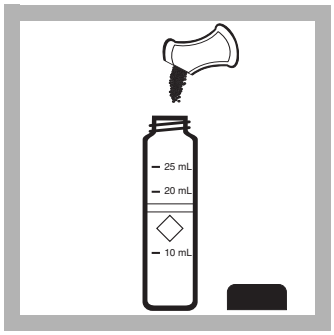
3. Add the contents of one FerroMo Iron Reagent 1 Powder Pillow to the graduated mixing cylinder. Stopper.



4. Invert several times to dissolve the reagents (the prepared sample).

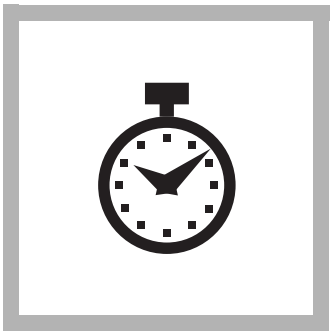


5. Fill a clean, round sample cell to the 25-mL mark with prepared sample. Save the remaining 25 mL of prepared sample for step 8.



6. Add the contents of one FerroMo Iron Reagent 2 Powder Pillow to the sample cell. Swirl to dissolve the reagents. This is the developed sample. A blue color will develop if iron is present.

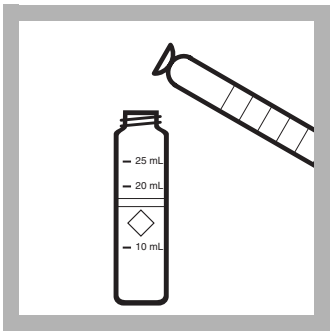
A small amount of undissolved reagent will not affect the results.



7. Touch the timer icon.

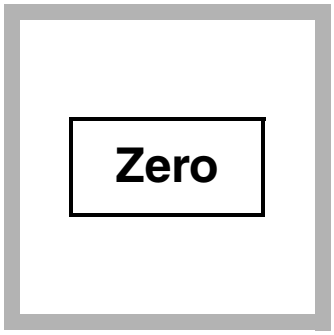
Touch **OK**.

A three-minute reaction period will begin.



8. Fill a second round sample cell with the remaining 25 mL of prepared sample from step 5 (the blank).

9. When the timer beeps, insert the blank into the cell holder.

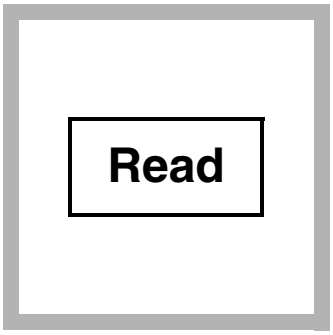


10. Touch **Zero**.

The display will show:

0.00 mg/L Fe

11. Place the developed sample into the cell holder.



12. Touch **Read**.

Results will appear in mg/L Fe.

Interferences

Interfering Substance	Interference Levels and Treatments
pH	A sample pH of less than 3 or greater than 4 after the addition of reagent may inhibit color formation, cause the developed color to fade quickly, or result in turbidity. Adjust the sample pH in the graduated cylinder before the addition of reagent to between 3 and 8 by using a pH meter or pH paper and adding, dropwise, an appropriate amount of iron-free acid or base such as 1.0 N Sulfuric Acid Standard Solution (Cat. No. 1270-32) or 1.0 N Sodium Hydroxide Standard Solution (Cat. No. 1045-32). Make a volume correction if significant volumes of acid or base are used; see *Section 3.1.3 Correcting for Volume Additions* on page *29.*

Sample Collection, Storage and Preservation

Collect samples in acid-washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with hydrochloric acid (about 2 mL per liter) (Cat. No. 134-49). Samples preserved in this manner can be stored up to six months at room temperature. If only dissolved iron is to be reported, filter sample immediately after collection through a 0.45-micron filter or equivalent medium before adding hydrochloric acid.

Before testing, adjust the sample pH to 3–5 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-32). Do not exceed pH 5 as iron may precipitate. Correct test results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open an Iron Voluette® Ampule Standard, 50-mg/L Fe.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-41) with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. The iron concentration should increase by 0.10 mg/L. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Using Class A glassware, prepare a 0.40-mg/L iron standard solution by pipetting 4.00 mL of Iron Standard Solution, 100-mg/L, into a 1-liter volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the iron procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 0.40-mg/L Fe Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust: Off**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 1.00 mg/L Fe

Program	95% Confidence Limits of Distribution
275	0.97–1.03 mg/L Fe

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.01 mg/L Fe

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

FerroMo Iron Reagent 1 contains a reducing agent combined with a masking agent. The masking agent eliminates interference from high levels of molybdate. The reducing agent converts precipitated or suspended iron, such as rust, to the ferrous state. FerroMo Iron Reagent 2 contains the indicator combined with a buffering agent. The indicator reacts with ferrous iron in the sample, buffered between pH 3 and 5, resulting in a deep blue-purple color. Test results are measured at 590 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
FerroMo® Iron Reagent Set (100 tests)			25448-00
Includes:			
(4) FerroMo® Iron Reagent 1 Powder Pillows	1 pillow	25/pkg	25437-68
(2) FerroMo® Iron Reagent 2 Powder Pillows	1 pillow	50/pkg	25438-66

Required Apparatus

Cylinder, graduated mixing, 50-mL, w/stopper	1	each	1896-41
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Iron Standard Solution, 100-mg/L		100 mL	14175-42
Iron Standard Solution, 10-mL Voluette® ampule, 50-mg/L Fe		16/pkg	14254-10
Water, deionized		4 liters	272-56

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 8317

LeadTrak®* Fast Column Extraction Method (5 to 150 µg/L)

Scope and Application: For drinking water

* Patent Number 5,019,516

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- The sampling requirements for "first-draw" analysis are detailed in *Sample Collection, Storage and Preservation on page 4*.
- Reagents will stain the sample cells, rinse the cells with 1:1 HNO_3, followed by deionized water.

Fast Column Extraction Method 8317

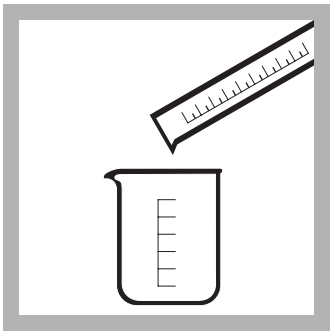
1. Touch

Hach Programs.

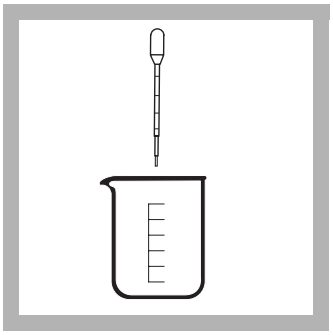
Select program

283 Lead, LeadTrak LR.

Touch **Start**.

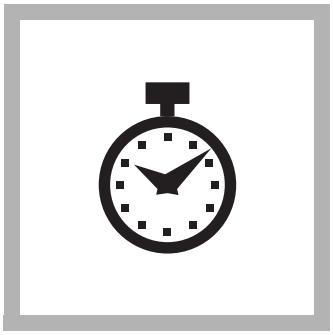


2. Fill a 100-mL plastic graduated cylinder with 100 mL of the sample. Pour the measured sample into a 250-mL plastic beaker.



3. Using a plastic 1-mL dropper, add 1.0 mL of pPb-1 Acid Preservative Solution to the sample and swirl to mix.

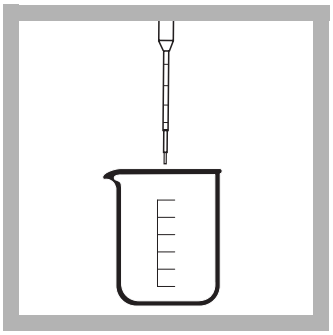
If the sample has been preserved previously with pPb-1 Acid Preservative at a ratio of 1.0 mL per 100 mL sample, omit steps 3 and 4.



4. Touch the timer icon.

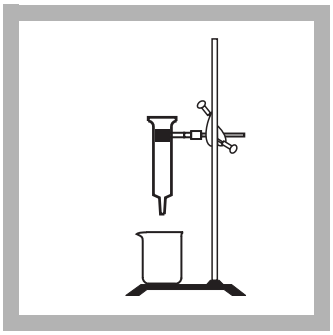
Touch **OK**.

A two-minute reaction period will begin.



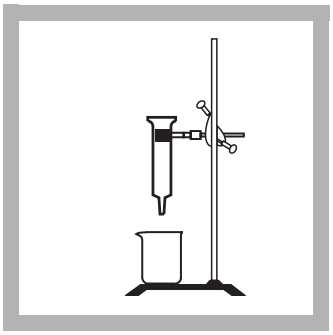
5. When the timer beeps, use a second 1-mL plastic dropper to add 2.0 mL of pPb-2 Fixer Solution. Swirl to mix.

Note: *Field samples that have been preserved with nitric acid or samples that have been digested may exceed the buffer capacity of the Fixer Solution. After step 5, check the pH of these samples and adjust with 5 N Sodium Hydroxide to a pH of 6.7–7.1 before proceeding with step 6.*



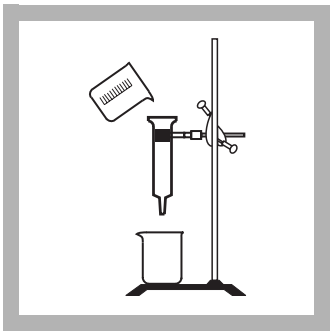
6. Mount a new Fast Column Extractor in a ring stand with a clamp. Place a 150-mL plastic beaker under the Extractor.

A Fast Column Extractor is included in the LeadTrak® Reagent Set. A new extractor is required for each test.



7. Soak the cotton plug with deionized water and compress it with the plunger. Remove the plunger. If the cotton plug moves up the column, push it back to the bottom with a clean, blunt rod.

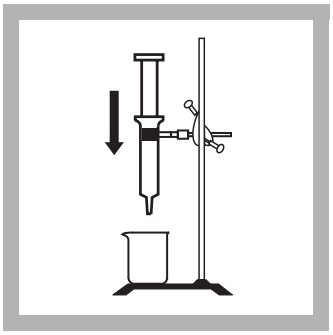
The cotton plug should fit snugly against the inner wall of the column.



8. Pour the prepared sample slowly into the center of the Column Extractor. Wait for the sample to flow through.

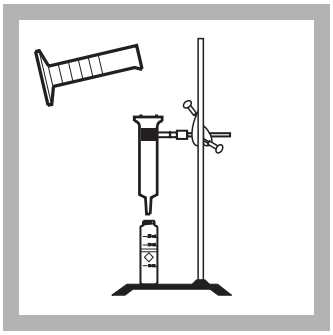
The sample solution should flow relatively slowly (2 drops per second) through the column.

Keep the level of the sample solution just above the cotton plug.



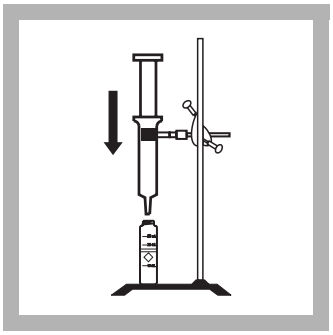
9. After the flow has stopped, fully compress the absorbent pad in the Extractor with the plunger. Discard the contents of the beaker. Slowly withdraw the plunger from the Extractor.

Note: *The absorbent pad should remain at the bottom of the Extractor when the plunger is removed. If the cotton plug moves up the column, push it back to the bottom with a clean, blunt rod.*



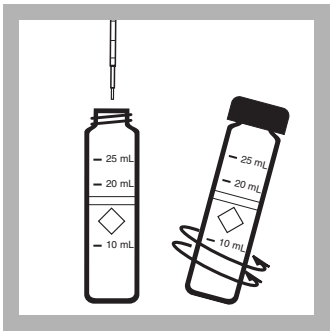
10. Place a clean, dry 25-mL sample cell under the Extractor. Using a 25-mL plastic graduated cylinder, add 25 mL of pPb-3 Eluant Solution to the Extractor.

Keep the level of the eluent solution just above the absorbent pad.

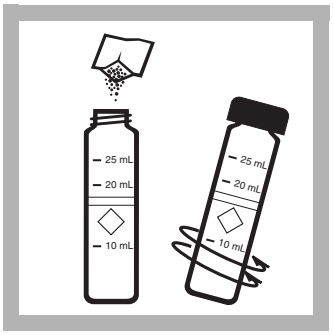


11. Allow the Eluant Solution to drip slowly from the Extractor.

After the flow has stopped, fully compress the absorbent pad. The volume in the sample cell should be 25 mL.

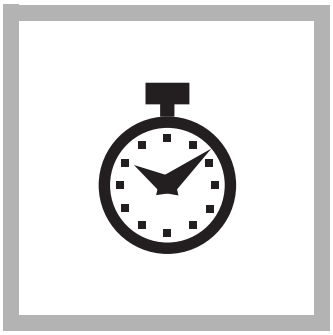


12. Using a 1-mL plastic dropper, add 1.0 mL of pPb-4 Neutralizer Solution to the cell. Swirl thoroughly to mix and proceed immediately to *step 13*.



13. Add the contents of one pPb-5 Indicator Powder Pillow to the sample and swirl thoroughly to mix.

The solution will turn brown.

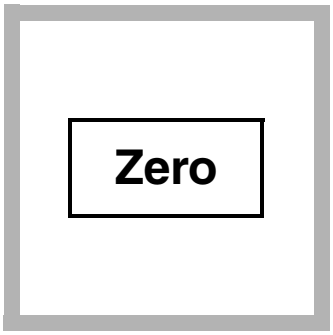


14. Touch the timer icon.

Touch **OK**.

A second two-minute reaction period will begin.

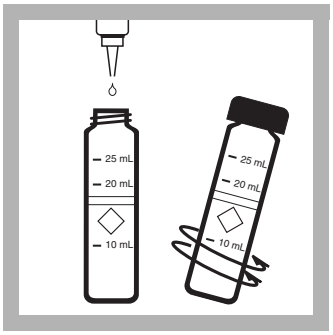
15. When the timer beeps, place the sample cell into the cell holder.



16. Touch **Zero**.

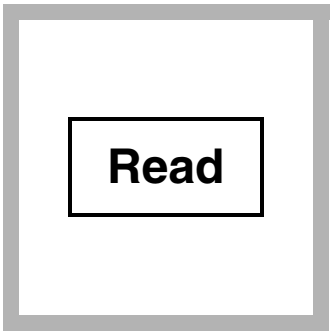
The display will show:

0 µg/L Pb



17. Remove the sample cell and add 6 drops of pPb-6 Decolorizer Solution to the cell. Swirl to mix thoroughly.

18. Place the sample cell into the cell holder.



19. Touch **Read**.

Results will appear in µg/L Pb.

Interferences

Interference studies were conducted by preparing a known lead solution of approximately 25 µg/L as well as the potential interfering ion. The ion was said to interfere when the resulting lead concentration changed by ±10%. Samples containing levels exceeding these concentration values may be diluted 1:1 and re-analyzed. Multiply the value obtained by a factor of 2 to determine the lead present in the original sample.

Every effort has been made to prevent contamination in packaging the reagents. Use of black rubber stoppers, black dropper bulbs and droppers with inked graduations may contaminate the sample and should be avoided. Use the plastic droppers provided in the reagent set.

Acid-wash all glassware and plasticware to prevent sample contamination, especially if the previous sample had a high lead level (see *Apparatus and Sample Preparation*).

The Extractor plunger may be reused for more than one test but should be rinsed between uses.

Interfering Substance	Interference Levels and Treatments
Aluminum, Al^{3+}	0.5 mg/L
Ammonium, NH_4^+	500 mg/L
Barium, Ba^{2+}	6 mg/L
Calcium, Ca^{2+}	500 mg/L
Chloride, Cl^-	1000 mg/L
Copper, Cu^{2+}	2 mg/L
Fluoride, F^-	10 mg/L
Iron, Fe^{2+}	2 mg/L
Magnesium, Mg^{2+}	500 mg/L
Manganese, Mn^{2+}	05 mg/L
Nitrate, NO_3^-	1000 mg/L
Sulfate, SO_4^{2-}	1000 mg/L
Zinc, Zn^{2+}	1 mg/L

Apparatus and Sample Preparation

Because lead is very common to our environment, care must be taken to prevent sample contamination. Follow these steps for greatest test accuracy:

- Lead-free water is necessary to minimize sample contamination when rinsing apparatus or diluting sample. The water may be either distilled or deionized. If the water is obtained from a grocery store, verify the lead concentration is zero from the label. If the lead concentration is uncertain, determine the lead concentration with the LeadTrak test.
- Plastic or glass sample containers and lids may be checked for contamination by rinsing with 1 mL of pPb-1 Acid Preservative Reagent (Cat. No. 23685-31). Add 100 mL of lead-free water. After 24 hours, analyze this solution using the LeadTrak test to confirm the absence of lead.
- Rinse glassware used in this test with a small amount of dilute lead-free 0.1 N nitric acid or pPb-1 Acid Preservative Reagent followed by rinsing with lead-free water.
- pPb-5 Indicator may be rinsed from the glass sample cells with a few drops of pPb-1 Acid Preservative Reagent or a small amount of dilute lead-free nitric acid.
- Acidify solutions containing lead with Nitric Acid (Cat. No. 152-49) or pPb-1 to below pH 2 to prevent adsorption of lead onto the container walls. See *Sample Collection, Storage and Preservation*.

Sample Collection, Storage and Preservation

Samples may be collected either from household pipes (point-of-use) or from water sources. Preserved samples may be stored up to six months. Each sample type typically requires different sampling procedures. Consult with the appropriate regulatory agency in your area for more information about your specific sampling requirements.

Sampling for Lead Contamination in Household Pipes for Point-of-use Drinking Water

- The sample should be collected after sitting in pipes with no flow for 8 to 18 hours.
- Add 10 mL of pPb-1 Acid Preservative (Cat. No. 23685-31) to a one-liter bottle.
- Turn on tap and collect exactly the first liter of water in the bottle containing acid preservative.
- Cap and invert several times to mix.
- After two minutes the sample is ready for analysis. Steps 3 and 4 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in step 5.

Sampling for Lead Contamination from Drinking Water Sources Such as Well Water or Water from Main Supply Lines

- Add 10 mL of pPb-1 Acid Preservative (Cat. No. 23685-31) to a one-liter bottle.
- Turn on the tap for 3–5 minutes or until the water temperature has been stable for 3 minutes.
- Collect exactly one liter of water into the bottle containing the acid preservative.
- Cap and invert several times to mix.
- After two minutes the sample is ready for analysis. Steps 3 and 4 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in step 5.
- At least one liter should be collected to obtain a representative sample. If less than one liter is collected, use 1 mL of pPb-1 Acid Preservative per 100 mL of sample.
- If nitric acid is to be substituted for pPb-1 as a preservative or the sample is digested, the buffering capacity of the pPb-2 Fixer Solution (Cat. No. 23686-55) may be exceeded. Adjust the sample pH to 6.7–7.1 pH with 5 N Sodium Hydroxide (Cat. No. 2450-53) after step 6.

Reagent Blank Adjustment

The LeadTrak® program will allow a reagent blank value from -5 to +5 µg/L Pb to be automatically subtracted from the test result. When using the reagent blank adjustment feature, the concentration value displayed after zeroing should be 0 µg/L.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.

4. Open a container of 10-mg/L (10,000 µg/L) Lead Standard Solution.
5. Prepare three sample spikes. Fill three beakers with 100 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Using Class A glassware, prepare a 100-µg/L lead working standard solution by pipetting 1.0 mL of Lead Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Use a TenSette® Pipet to add 0.2 mL of concentrated nitric acid to the flask. Dilute to the mark with lead-free deionized water. This makes a 10-mg/L working standard.

Pipet 10.00 mL of this working solution into a 1-liter plastic volumetric flask. Add 2.0 mL of concentrated nitric acid to the flask. Dilute to the mark with lead-free water. This 100-µg/L standard solution should be prepared immediately before use. Perform the LeadTrak® procedure as described above.

Alternatively, prepare a 100-µg/L lead standard solution by using a TenSette® Pipet to pipet 0.2 mL from a Lead Voluette® Ampule Standard Solution, 50-mg/L as Pb, into a 100-mL plastic volumetric flask. Add 0.2 mL of concentrated nitric acid, and dilute to volume with deionized water. Prepare this solution immediately before use.

To adjust the calibration curve using the reading obtained with the 100-µg/L standard solution:

1. Touch **Options** on the current program menu. Touch **Standard Adjust**.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 750.0 µg/L Pb^{2+}

Program	Standard Deviation of 7 Replicate Standards
283	10.15 µg/L Pb^{2+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
50 to 150 µg/L	0.010	16 µg/L Pb^{2+}
0.010	0.010	1.9 µg/L Pb^{2+}
80	0.010	1.4 µg/L Pb^{2+}
106	0.010	1.3 µg/L Pb^{2+}
132	0.010	1.4 µg/L Pb^{2+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Acid soluble lead, as Pb^{2+}, in a potable water sample is first concentrated on a Fast Column Extractor. The lead is then eluted from the Extractor and determined colorimetrically with an indicator. Test results are measured at 477 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
LeadTrack® Reagent Set	1	20 tests/pkg	23750-00

Required Apparatus

Beaker, polypropylene, 150-mL	1	each	1080-44
Beaker, polypropylene, 250-mL	1	each	1080-46
Clamp, two-prong extension	1	each	21145-00
Clamp holder	1	each	326-00
Clippers, for opening powder pillows	1	each	936-00
Cylinder, graduated, polypropylene, 25-mL	1	each	1081-40
Cylinder, graduated, polypropylene, 100-mL	1	each	1081-42
Dropper, 0.5 & 1.0 mL marks		20/pkg	21247-20
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Support, ring stand	1	each	563-00

Digestion Reagents and Required Standards

Lead Standard Solution, 1000-mg/L as Pb	100 mL	12796-42
Lead Standard Solution, 50-mg/L, 10-mL Voluette® Ampules	16/pkg	14262-10
Lead Standard Solution, 10-mg/L	25 mL	23748-20
Nitric Acid, ACS	500 mL	152-49
Water, deionized	4 liters	272-56

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FAX: (970) 669-2932

3/04 1ed

Lead_None_Other_LTK_Eng_Ody.fm

Lead

PAR Method

(0.20 to 2.00 mg/L)

UniCell™ Vials

Scope and Application: For wastewater process control

Tips and Techniques

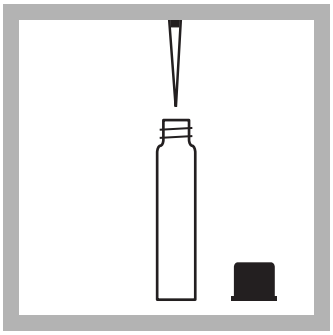
- Adjust pH of preserved samples to between pH 3–5 before analysis.
- Digest water samples with a pH higher than 9 before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check*.
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure that the temperature of the sample and the reagents is between 15–25 °C.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

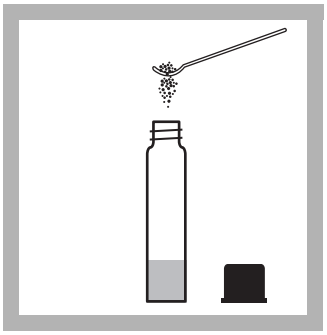
1. Touch **Hach Programs**.

Select program **286 Lead, HCT 152**.

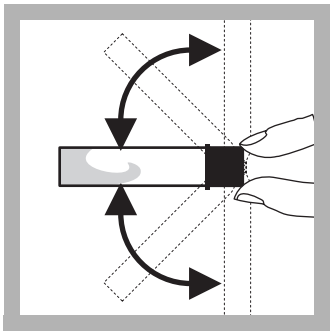
Touch **Start**.



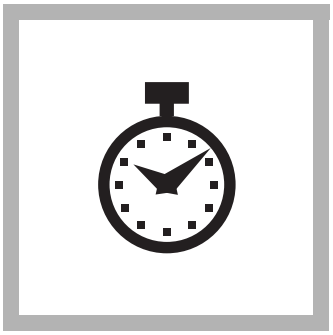
2. Pipet 10.0 mL of sample into the reaction tube (red cap).



3. Use the spoon to add one level spoonful of Masking Agent A (HCT 152 A) to the reaction tube.

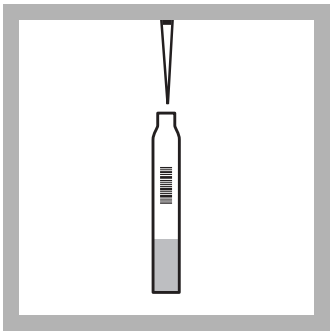


4. Close the reaction tube and invert several times to mix.

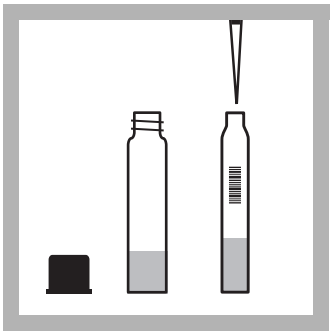


5. Touch the timer icon.

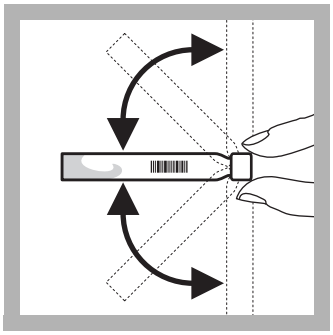
Touch **OK**.
A two-minute reaction period will begin.



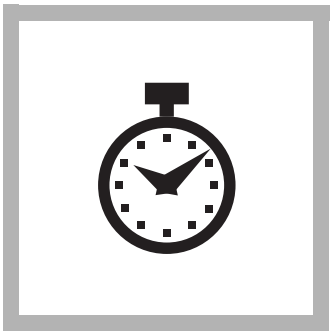
6. Pipet 1.5 mL of Buffer Solution B (HCT 152 B) into a sample vial (**light red** cap).



7. Pipet 4.0 mL of sample from the reaction tube into the sample vial.



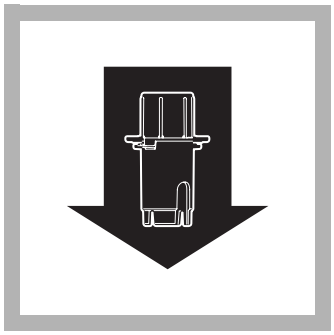
8. Close the sample vial and invert several times to mix.



9. Touch the timer icon.

Touch **OK**.

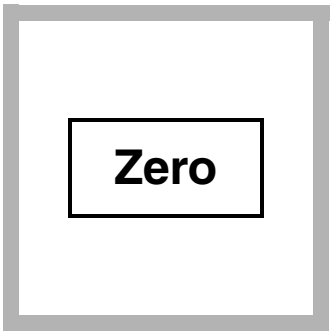
A second two-minute reaction period will begin.



10. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

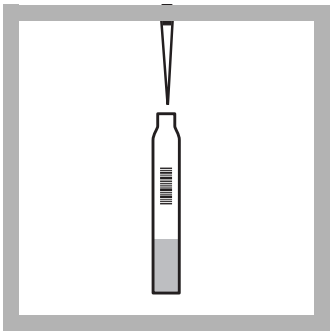
When the timer beeps, wipe the sample vial and place it into the cell adapter.



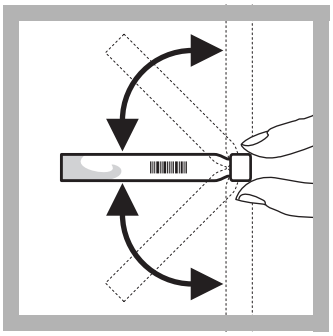
11. Touch **Zero**.

The display will show:

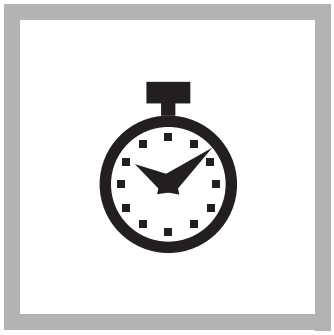
0.00 mg/L Free Pb
Underrange



12. Pipet 0.3 mL of Masking Solution C (HCT 152 C) into the sample vial.



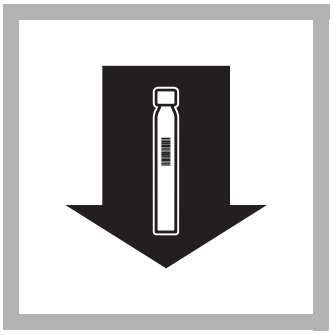
13. Close the sample vial and invert several times to mix.



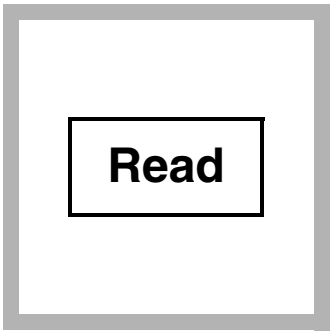
14. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin.



15. Wipe the sample vial and place it into the celladapter.



16. Touch **Read**.

Results will appear in mg/L Free Pb.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Ca^{2+}, Mg^{2+}, NO_3^-, Cl^-, PO_4^{3-}, CO_3^{2-}	500 mg/L
F^-, NH_4^+, Sr^{2+}	50 mg/L
Ag^+, Cd^{2+}, Cr^{6+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}	25mg/L
Cr^{3+}, Al^{3+}, Fe^{2+}, Fe^{3+}	10 mg/L
Mn^{2+}, Hg^{2+}	5 mg/L
Sn^{2+}	0.5 mg/L

Total lead, including undissolved lead hydroxide and complexed lead, can only be determined after digesting with the Metal Prep Set, HCT 200.

Note: *The total lead measuring range is 0.24–2.40 mg/L.*

Sample Collection, Storage and Preservation

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3 and 5 with 5.0 N Sodium Hydroxide Standard Solution. Water samples which are free from complexing agents and organic compounds can be analyzed directly. Other water samples have to be digested with the Metal Prep Set in order to bring undissolved lead hydroxide or complex lead compounds into solution.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
2. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
3. Prepare three sample spikes. Fill three mixing cylinders with 100 mL of sample. Use a pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of 100-mg/L Pb standard, respectively, to each sample and mix thoroughly.
4. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
5. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 1.00 mg/L Pb standard solution by pipetting 1.0 mL of 100-mg/L Pb into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the Lead procedure as described.

To adjust the calibration curve using the reading obtained with the 1.0-mg/L standard solution:

1. Touch **Options** on the current program menu. Touch **Standard Adjust**.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 1.00 mg/L Free Pb

Program	95% Confidence Limits of Distribution
286	0.76–1.24 mg/L Free Pb

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
286	Entire range	0.010	0.08 mg/L Free Pb

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Lead (II) ions react at pH 9 with 4-(2-pyridylazo)-resorcinol (PAR) to form a red complex. Measurements are taken at 520 nm.

Required Reagents

Description	Unit	Cat. No.
Lead - Pb, UniCell™ HCT 152	24/pkg	HCT 152

Optional Reagents

Lead Standard Solution, 100-mg/L as Pb	100 mL	12617-42
Metal Prep Set	50 digestions	HCT 200

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Flask, volumetric, 100-mL, Class A	each	14574-42
Graduated cylinder, mixing, 100-mL	each	20886-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00
Pipettor, (Jencons) 100–1000 µL	1 each	27949-00
Replacement tips for 27949-00	400/pkg	27950-00
pH Paper	100/pkg	26013-00

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★Method 8034 Periodate Oxidation Method*

Powder Pillows HR (0.2 to 20.0 mg/L)

Scope and Application: For soluble manganese in water and wastewater; USEPA approved for reporting wastewater analyses (digestion required)**

* Adapted from *Standard Methods for the Examination of Water and Wastewater*
** *Federal Register,* 44(116) 34193 (June 14, 1979)

Tips and Techniques

- Digestion required. See *2.3 Digestion* in the Laboratory Practices section of the DR/2400 Procedures Manual.
- If only dissolved manganese is to be determined, filter the sample before acid addition.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.

Powder Pillows Method 8034

1. Touch

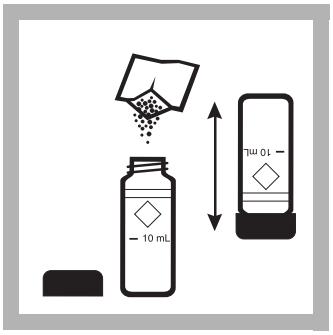
Hach Programs.

Select program

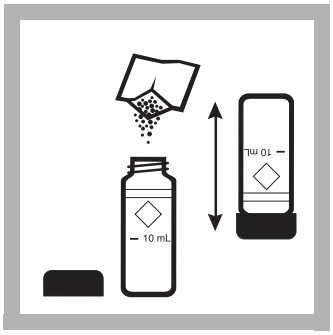
295 Manganese HR.

Touch **Start**.

2. Fill a round sample cell with 10 mL of sample.

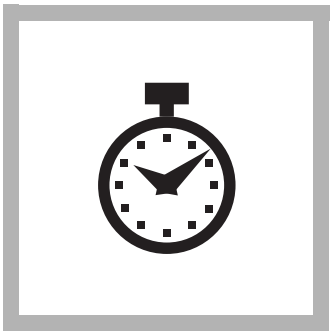


3. Add the contents of one Buffer Powder Pillow, Citrate Type for Manganese. Cap and invert gently to mix.



4. Add the contents of one Sodium Periodate Powder Pillow to the sample cell. Cap and invert gently to mix.

A violet color will develop if manganese is present.



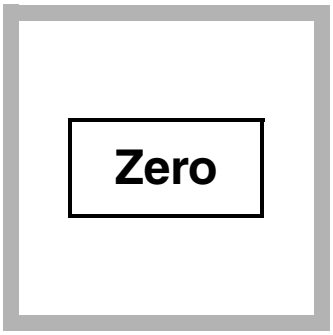
5. Touch the timer icon.

Touch **OK**.

A two-minute reaction period will begin.

6. Fill another round sample cell with 10 mL of sample.

7. When the timer beeps, place the blank into the cell holder.

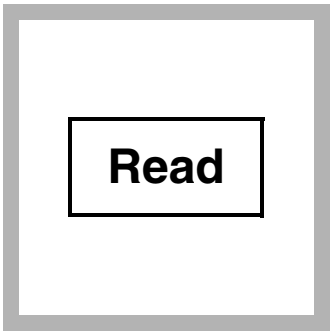


8. Touch **Zero**.

The display will show:

0.0 mg/L Mn

9. Within eight minutes of the timer beep, place the sample into the cell holder.



10. Touch **Read**.

Results will appear in mg/L Mn.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	700 mg/L
Chloride	70,000 mg/L
Iron	5 mg/L
Magnesium	100,000 mg/L
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment; see *Section 3.3 Interferences* on page *41.*

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Do not use glass containers due to possible adsorption of Mn to glass. If samples are acidified, adjust the pH to 4–5 with 5.0 N Sodium Hydroxide (Cat. No. 2450-32) before analysis. Do not exceed pH 5, as manganese may precipitate. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Manganese Voluette® Ampule Standard, 250-mg/L Mn (Cat. No. 14258-10).
5. Prepare three sample spikes. Fill three Graduated Mixing Cylinders (Cat. No. 1896-40) with 10 mL of sample. Use the TenSette® Pipet (Cat. No. 19700-01) to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly. This will result in a 2.5 mg/L increase in manganese in each sample spike.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 10.0-mg/L manganese standard solution by pipetting 10.0 mL of Manganese Standard Solution, 1000-mg/L, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the manganese periodate oxidation procedure as described above.
2. The calibration curve can be adjusted to account for variability in laboratory technique. To adjust the calibration curve using the reading obtained with the 10.0 mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision
Standard: 10.0 mg/L Mn

Program	95% Confidence Limits of Distribution
295	9.8–10.2 mg/L Mn

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
0.010 abs	0.010	0.13 mg/L Mn
10 mg/L	0.010	0.14 mg/L Mn
18 mg/L	0.010	0.15 mg/L Mn

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Manganese in the sample is oxidized to the purple permanganate state by sodium periodate, after buffering the sample with citrate. The purple color is directly proportional to the manganese concentration. Test results are measured at 525 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
High Range Manganese Reagent Set (100 Tests)			24300-00
Includes:			
Buffer Powder Pillows, citrate type for manganese	1 pillow	100/pkg	21076-69
Sodium Periodate Powder Pillows, for manganese	1 pillow	100/pkg	21077-69

Required Apparatus

Sample Cells, 10 mL, w/cap	2	6/pkg	24276-06

Required Standards

Manganese Standard Solution, 1000-mg/L Mn		100 mL	12791-42
Manganese Standard Solution, 10-mL Voluette® Ampule, 250-mg/L Mn		16/pkg	14258-10
Water, deionized		4 liters	272-56

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Method 8149 **1-(2-Pyridylazo)-2-Naphthol PAN Method***

Powder Pillows **LR (0.007 to 0.700 mg/L)**

Scope and Application: For water and wastewater; digestion is required for determining total manganese (see *Section 2* for digestion procedure)

* Adapted from Goto, K., et al., *Talanta*, 24, 652-3 (1977)

Tips and Techniques

- Rinse all glassware with 1:1 Nitric Acid Solution. Rinse again with deionized water.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- The alkaline cyanide solution contains cyanide. Cyanide solutions should be collected for disposal as a reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See *Section Section 4 Waste Management and Safety* on page *55* for more information on proper disposal of these materials.

Powder Pillows Method 8149

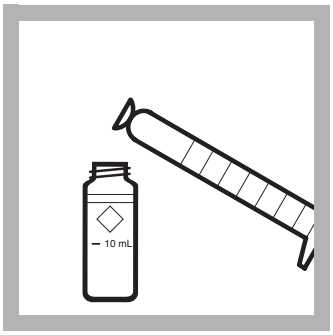
1. Touch

Hach Programs.

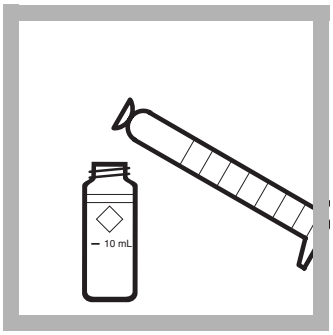
Select program

290 Manganese LR.

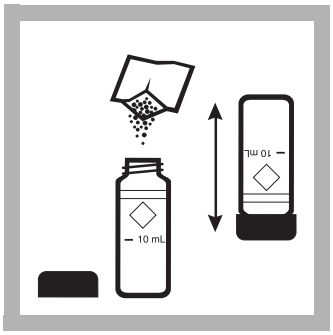
Touch **Start**.



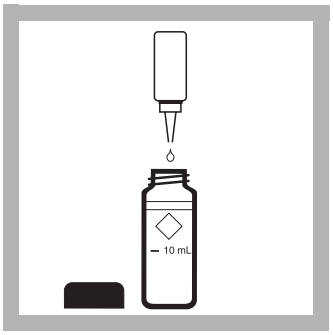
2. Pour 10.0 mL of deionized water into a round sample cell (the blank).



3. Pour 10.0 mL of sample into another round sample cell (the prepared sample).

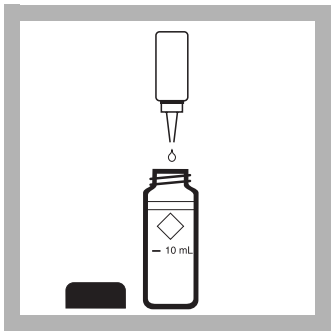


4. Add the contents of one Ascorbic Acid Powder Pillow to each cell. Cap and invert gently to mix.



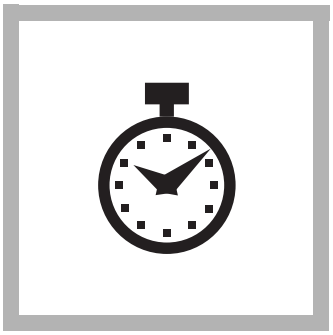
5. Add 15 drops of Alkaline-Cyanide Reagent Solution to each cell. Cap and invert gently to mix.

A cloudy solution may form. The turbidity should dissipate after *step 6*.



6. Add 21 drops of PAN Indicator Solution, 0.1%, to each sample cell. Cap and invert gently to mix.

An orange color will develop in the sample if manganese is present.

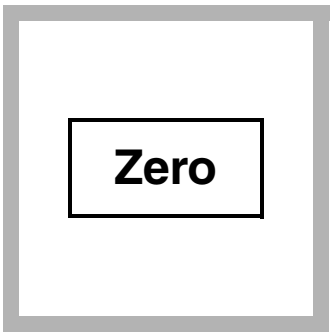


7. Touch the timer icon.

Touch **OK**.

A two-minute reaction period will begin.

8. When the timer beeps, wipe the blank and place it into the cell holder.

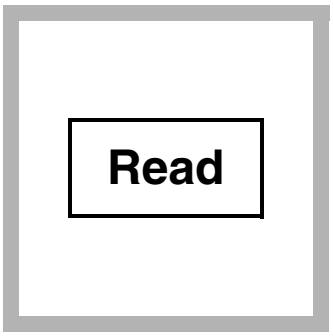


9. Touch **Zero**.

The display will show:

0.000 mg/L Mn

10. Wipe the prepared sample and place it into the cell holder.



11. Touch. **Read**.

Results will appear in mg/L Mn.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	20 mg/L
Cadmium	10 mg/L
Calcium	1000 mg/L as $CaCO_3$
Cobalt	20 mg/L
Copper	50 mg/L
Iron	25 mg/L (If sample contains more than 5 mg/L iron, allow a 10-minute reaction period in *step 7*.)
Lead	0.5 mg/L
Magnesium	300 mg/L as $CaCO_3$
Nickel	40 mg/L
Zinc	15 mg/L

For samples that contain hardness greater than 300 mg/L $CaCO_3$, add 10 drops of Rochelle Salt Solution (Cat. No. 1725-33) to the sample **after** adding the Ascorbic Acid Powder Pillow in step 4.

Sample Collection, Storage and Preservation

Collect samples in a clean plastic container. Adjust the pH to 2 or less with Concentrated Nitric Acid (about 2 mL per liter) (Cat. No. 152-49). Preserved samples can be stored up to six months at room temperature. Adjust the pH to between 4–5 with 5.0 N Sodium Hydroxide (Cat. No. 2450-32) before analysis. Correct the test result for volume additions; see *3.1.3 Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Manganese Voluette® Ampule Standard, 10-mg/L Mn.
5. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 10 mL of sample. Use the TenSette® Pipet (Cat. No. 19700-01) to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 0.5-mg/L manganese standard solution by pipetting 2.0 mL of Manganese Voluette Standard Solution, 250-mg/L Mn, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. This solution should be prepared daily. Perform the manganese procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 0.5-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 0.500 mg/L Mn

Program	95% Confidence Limits of Distribution
290	0.492–0.508 mg/L Mn

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.007 mg/L Mn

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The PAN method is a highly sensitive and rapid procedure for detecting low levels of manganese. An ascorbic acid reagent is used initially to reduce all oxidized forms of manganese to Mn^{2+}. An alkaline-cyanide reagent is added to mask any potential interferences. PAN Indicator is then added to combine with the Mn^{2+} to form an orange-colored complex. Test results are measured at 560 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Manganese Reagent Set, 10-mL (50 tests)			26517-00
Includes:			
Alkaline Cyanide Reagent	30 drops	50 mL SCDB	21223-26
Ascorbic Acid Powder Pillows	2 pillows	100/pkg	14577-99
PAN Indicator Solution, 0.1%	42 drops	50 mL SCDB	21224-26
Water, deionized	10 mL	4 liters	272-56

Required Apparatus

Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

OPTIONAL REAGENTS AND STANDARDS

Manganese Standard Solution, 10-mg/L Mn, 2-mL ampule		20/pkg	26058-20
Manganese Standard Solution, 250-mg/L Mn, 10-mL Voluette® ampule		16/pkg	14258-10

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 10065 Cold Vapor Mercury Concentration Method* (0.1 to 2.5 µg/L)

Scope and Application: For water, wastewater, and seawater

* Patent no. 5,733,786

Tips and Techniques

- Perform phase 1 of the procedure in a fume hood. Toxic chlorine or other gases may be produced.
- Use dedicated digestion glassware and sample cells for this procedure.
- Determine a reagent blank for each new lot of reagent by running the entire procedure, including the digestion, using one liter of deionized water instead of sample. Add the same amount of potassium permanganate as required by the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.

Phase 1: Sample Digestion

(Must be done in a fume hood – toxic gases may be produced!)

1. Transfer one liter of the sample to a 2000-mL Erlenmeyer flask. Add a 50-mm magnetic stir bar to the sample. Place the flask on a magnetic stirring hot plate and begin stirring.

2. Add 50 mL of concentrated sulfuric acid to the sample.

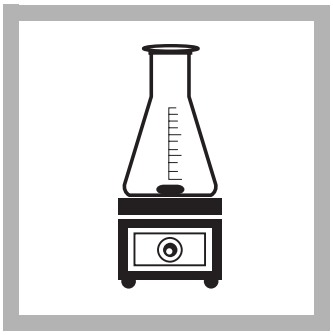
3. Add 25 mL of concentrated nitric acid to the sample.

4. Add 4.0 g of potassium persulfate to the sample. Stir until dissolved.

Alternatively, add one 5-gram measuring scoop of potassium persulfate to the sample.

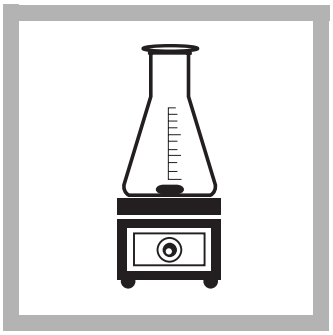
5. Add 7.5 g of potassium permanganate to the sample. Stir until dissolved.

Alternatively, add a 10-gram measuring scoop of potassium permanganate to the sample.



6. Cover the flask with a watch glass. Begin heating the sample to a temperature of 90 °C after the reagents have dissolved. **Do not boil.**

Note: *For a mercury standard or reagent blank in distilled water, the heat step is not necessary.*

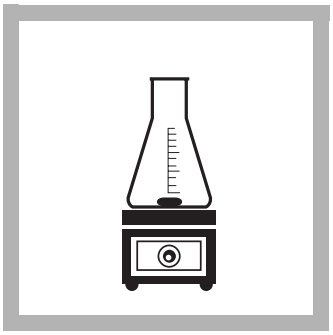


7. Continue to stir and heat the sample at 90 °C for two hours.

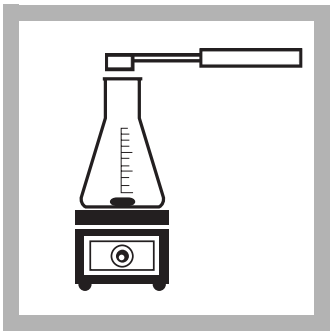
The solution must remain dark purple throughout the entire digestion. Some samples, such as sea waters, industrial effluents or other samples high in organic matter or chloride concentration, require additional permanganate. It may be difficult to see a dark purple color if the sample contains black/brown manganese dioxide precipitate. Add more potassium permanganate if the solution is not dark purple.

8. Cool the digested sample to room temperature.

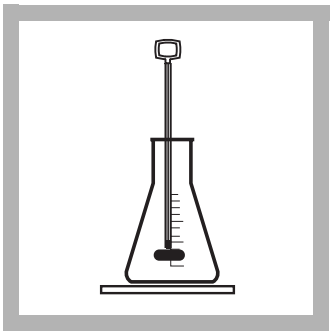
A brown/black precipitate of manganese dioxide may settle during cooling. If the digested sample does not have a purple color, the digestion may be incomplete. Add more potassium permanganate. Return the sample to the magnetic stirring hot plate and continue the digestion until the purple color persists.



9. Return the cool, digested sample to the cool, magnetic stirring hot plate. Turn on the stirrer.



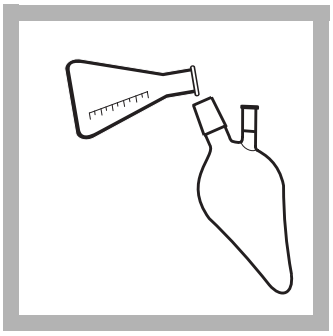
10. Using a 0.5-g measuring spoon, add 0.5 g additions of hydroxylamine-hydrochloride until the purple color disappears. Wait 30 seconds after each addition to see if the purple disappears. Add hydroxylamine-hydrochloride until all manganese dioxide is dissolved.



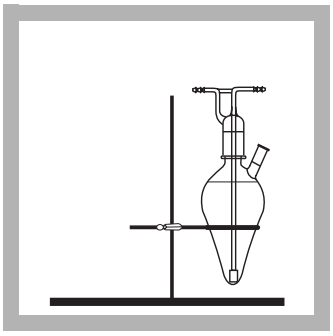
11. Remove the stir bar.

12. The digested sample is now ready for processing by cold vapor separation and preconcentration. Proceed to Phase 2.

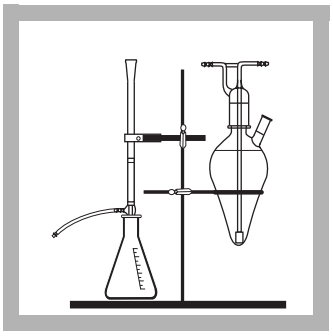
Phase 2: Cold Vapor Separation and Preconcentration of Mercury



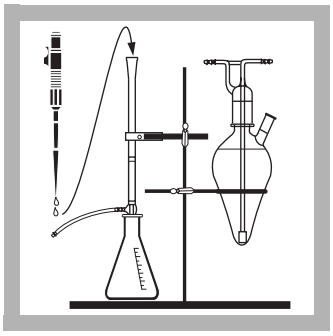
1. Transfer the digested sample to the Cold Vapor Gas Washing Bottle. (The volume of the digested sample should contain 0.1 to 2.5 µg Hg.)



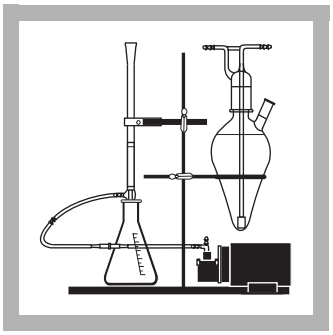
2. Set the Gas Washing Bottle in the support ring. Place the top on the Gas Washing Bottle. Wait until *step 9* to connect the mercury absorber column to the Gas Washing Bottle.



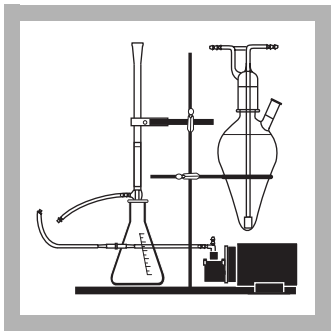
3. Connect the 100-mL Erlenmeyer flask to the mercury absorber column.



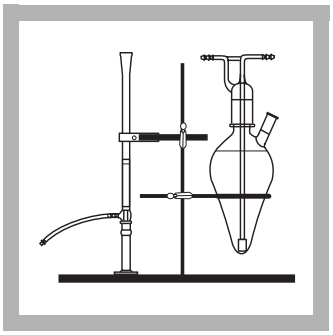
4. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber column.



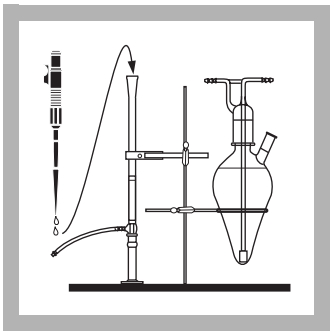
5. Connect the power to the vacuum pump and apply vacuum to the Mercury Absorber Column. Draw most of the HgEx Reagent B into the Erlenmeyer flask.



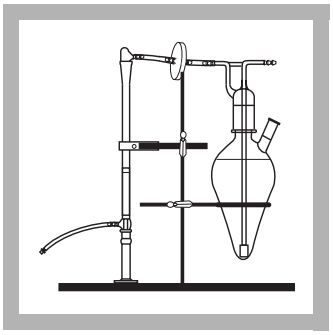
6. Disconnect the vacuum using the quick disconnect when HgEx Reagent B begins to drip from the inner delivery tube on the Mercury Absorber Column (about 10 seconds after starting the vacuum). Do not draw enough air through the column to begin drying the packing.



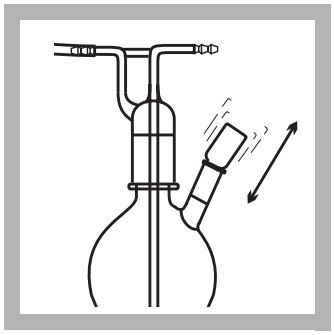
7. Remove the 100-mL Erlenmeyer flask from the Mercury Absorber Column. Replace it with the 10-mL Distilling Receiver.



8. Pipet 2 mL of HgEx Reagent C into the Mercury Absorber Column.

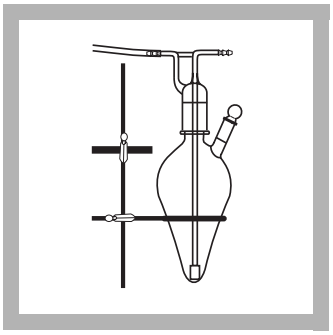


9. Connect the Mercury Absorber column to the Gas Washing Bottle using the glass elbow.

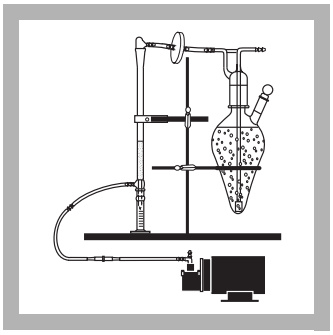


10. Shake an ampule of HgEx Reagent A to suspend undissolved reagent.

Open the ampule and gently shake the contents into the Gas Washing Bottle through the side neck.



11. Stopper the side neck on the Glass Washing Bottle.



12. Reconnect the vacuum to the Mercury Absorber Column using the quick disconnect. The vacuum will pull HgEx Reagent C through the Mercury Absorber Column packing and into the 10-mL receiver. Air bubbles should be produced at the gas dispersion tube in the Gas Washing Bottle. Perform steps 13–14 immediately.

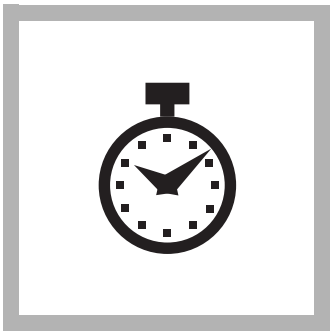
13. Touch

Hach Programs.

Select program

312 Mercury, Cold Vapor.

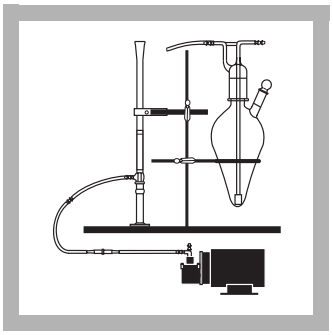
Touch **Start**.



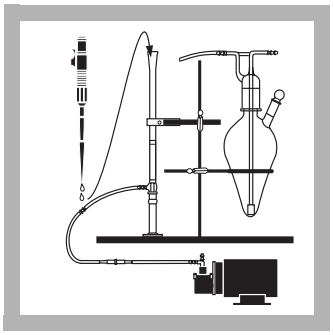
14. Touch the timer icon. Touch **OK**.

A five-minute reaction period will begin. Let the solution bubble for this period.

Air flow rate through the Gas Washing Bottle should be between 1–5 L/min. Allow more bubbling time for lower air flow rates. For example, if the air flow rate is 1 L/min., let the solution bubble for 10 min.

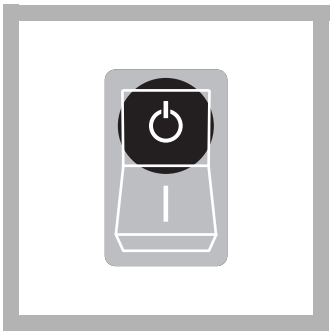


15. After the timer beeps, remove the glass elbow from the top of the Mercury Absorber Column. Keep the vacuum pump on.



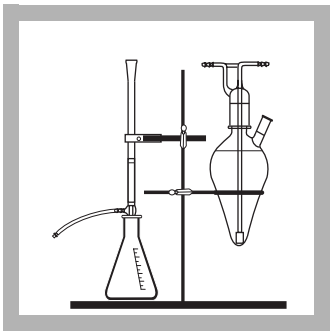
16. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber Column to elute the captured mercury.

Continue to apply vacuum to pull the HgEx Reagent B into the Distilling Receiver.

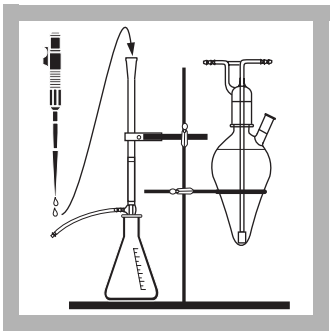


17. Turn off, or disconnect power to the vacuum pump when the volume in the Distilling Receiver reaches the 10 mL mark.

If necessary, the volume in the Distilling Receiver may be brought up to 10 mL with HgEx Reagent B. To avoid low volumes in the future, disconnect the vacuum a little sooner in step 6. This leaves more HgEx Reagent B in the packing of the Mercury Absorber Column.



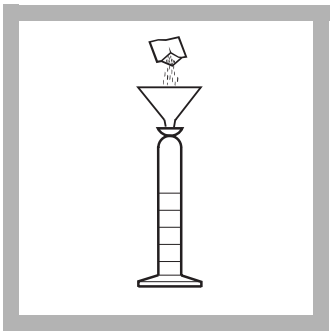
18. Remove the distilling Receiver from the Mercury Absorber Column. Reconnect the 100-mL Erlenmeyer flask to the column.



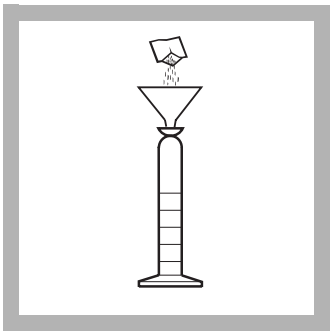
19. Pipet 3 mL of HgEx Reagent B into the Mercury Absorber Column without applying vacuum. This keeps the absorber packing wet between tests.

The Mercury Absorber Column eluate in the Distilling Receiver is ready for analysis. Proceed to Phase 3.

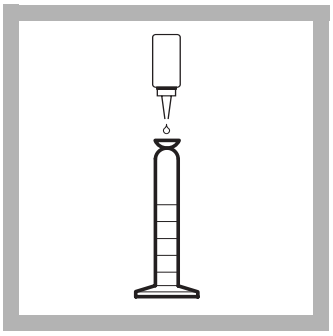
Phase 3: Colorimetric Analysis



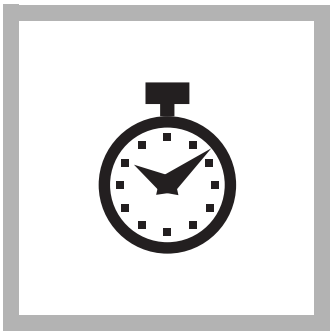
1. Using the funnel provided, add the contents of one HgEx Reagent 3 foil pillow to the eluate in the Distilling Receiver. Stopper the receiver. Invert to dissolve the reagent.



2. Add the contents of one HgEx Reagent 4 foil pillow to the Distilling Receiver using the funnel provided. Stopper the receiver. Invert to dissolve the reagent.



3. Add 8 drops of HgEx Reagent 5 to the Distilling Receiver. Stopper the Receiver. Invert to mix.



4. Touch the timer icon.

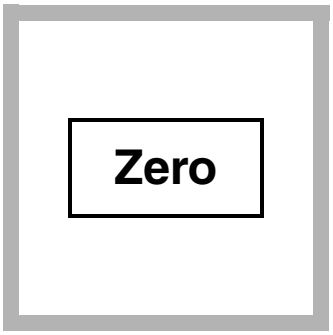
Touch **OK**.

A two-minute reaction period will begin.

5. During the reaction period, transfer the solution to a sample cell. Wipe the sample cell sides with a clean tissue.

Do not cap the sample cell.

6. After the timer beeps, place the prepared sample into the cell holder.



7. Touch **Zero**.

The display will show:

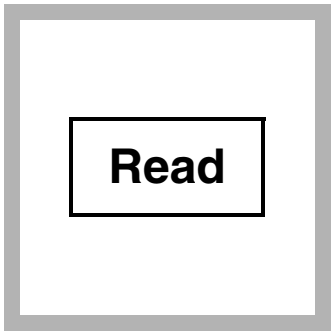
0.1 µg/L Hg

(This program uses a non-zero intercept.)

8. Remove the cell from the cell holder. Add the contents of one HgEx Reagent 6 foil pillow to the solution. Swirl or invert the cell until the reagent is completely dissolved. Immediately go to step 9.

Do not use the funnel to add HgEx Reagent 6 to the sample cell. Any HgEx Reagent 6 in the funnel will make mercury undetectable in subsequent tests.

9. Return the sample cell to the cell holder.



10. Touch **Read**.

Results will appear in µg/L Hg.

Interferences

Standards were used to prepare a single test solution with the following matrix. A second test solution containing only mercury at the same concentration was prepared as the control. The two solutions were digested then analyzed concurrently. There was no interference from the matrix of the test solution at the concentrations listed:

In addition, no interference occurred with a test solution containing 1000 mg/L Na^+, 1000 mg/L K^+, 1000 mg/L Mg^{2+}, and 400 mg/L Ca^{2+}.

Ion or Substance	Concentration
Ag^{+}	7 mg/L Ag^{+}
Al^{+3}	10 mg/L Al^{+3}
Au^{+3}	500 µg/L Au^{+3}
Cd^{+2}	10 mg/ L Cd^{+2}
Co^{+2}	10 mg/L Co^{+2}
Cr^{+6}	10 mg/L Cr^{+6}
Cu^{+2}	10 mg/L Cu^{+2}
F^{-}	1.0 mg/L F^{-}
Fe^{+2}	100 mg/L Fe^{+2}
Hg^{+2}	1 µg/L Hg^{+2}
Mo^{+6}	10 mg/L Mo^{+6}
Ni^{+2}	10 mg/L Ni^{+2}
NO_3^{-}–N	50 mg/L NO_3^{-}–N
Pb^{2+}	10 mg/L Pb^{2+}
SiO_2	100 mg/L SiO_2
Zn^{+2}	10 mg/L Zn^{+2}

Sample Collection and Preservation

Collect 1000 mL of sample in an analytically clean, glass or polyethylene terephthalate (PET) container. Add 10 mL of concentrated hydrochloric acid to preserve the sample before sample collection. Fill the container completely full to minimize air space when closed. Close a glass container with a ground glass stopper. Close a PET container with a PET cap or a polypropylene cap (no liner).

Store aqueous samples at 2–6 °C. Acid-preserved samples are stable for at least 6 months.

Accuracy Check

Standard Additions Method

1. Prepare a 10.0-mg/L Mercury Standard Solution as described under *Standard Solution Method, Step 3a,* below.
2. Use a TenSette® Pipet to add 0.10 mL of the 10.0-mg/L Mercury Standard Solution to the purged solution in the Gas Washing Bottle after an analysis has been performed. Immediately stopper the Gas Washing Bottle.
3. Begin at *step 3* of Phase 2. Follow the procedure steps.
4. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg.

Standard Solution Method

1. Transfer 800 mL of deionized water into the Gas Washing Bottle.
2. Add 50 mL of concentrated sulfuric acid and 25 mL of concentrated nitric acid to the water. Swirl to mix.

3. Prepare a 0.1-mg/L mercury standard solution by serially diluting a 1000-mg/L Mercury Standard Solution:
   a. To make a 10.0 mg/L standard, add 1.0 mL of concentrated nitric acid to a 500-mL volumetric flask. Dilute 5.00 mL of a 1000-mg/L standard to 500 mL with deionized water. Mix well.
   b. To make a 1.0-mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100-mL volumetric flask. Dilute 10.0 mL of the 10.0-mg/L standard to 100 mL with deionized water. Mix well.
   c. To make a 0.1-mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100-mL volumetric flask. Dilute 10.00 mL of the 1.0-mg/L solution to 100 mL with deionized water. Mix well.
4. Pipet 10.0 mL of the 0.1-mg/L mercury standard solution into the Gas Washing Bottle. Swirl to mix.
5. Begin at *step 2* of Phase 2. Follow the procedure steps.
6. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg.

System Start Up

Hach recommends that the analyst perform a few analyses on mercury standards and blanks for system equilibration before beginning sample testing. This allows the system to stabilize before processing samples.

Startup Standard

Test a mercury standard solution by following the procedure under *Accuracy Check* using the *Standard Solution Method*. Continue with *step 1* (below) if the value is not within specified limits.

1. Pipet 10.0 mL of the 0.1-mg/L mercury standard solution into the purged solution in the Gas Washing Bottle. Immediately stopper the Gas Washing Bottle.
2. Begin at *step 3* of Phase 2. Follow the procedure steps.
3. Test the eluate as described in Phase 3. The displayed concentration should be 0.9–1.1 µg/L Hg. Repeat *steps 1–3* if the value is not within these limits.

Startup Blank

Run a system blank by using the purged solution in the Gas Washing Bottle after a satisfactory test of the Startup Standard has been completed.

1. Leave the purged solution in the Gas Washing Bottle. Do not add an aliquot of mercury standard.
2. Begin at *step 3* of Phase 2. Follow the procedure steps.
3. Test the eluate as described in Phase 3. The displayed concentration should be ≤ 0.2 µg/L Hg. Repeat the *Startup Blank* procedure until a reproducible value is obtained.

Method Performance

Precision

Standard: 1.40 µg/L Hg

Program	95% Confidence Limits
312	1.3–1.5 µg/L Hg

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.03 µg/L Hg

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Storage and Maintenance of the Cold Vapor Mercury Apparatus

Storage

Store the apparatus as follows for fastest system stabilization and greatest sensitivity:

- Store the Gas Washing Bottle filled with deionized water containing 15 mL of concentrated sulfuric acid. Seal the bottle with the Gas Washing Bottle stopper and top.
- Store the Mercury Absorber Column with the packing wetted with HgEx Reagent B. The erlenmeyer flask should be kept attached underneath the column. The top of the Mercury Absorber column should be attached to the Gas Washing Bottle with the glass elbow as in the procedure.

Glassware Care

Hach recommends using dedicated glassware and sample cells because of the sensitivity of this procedure. Thoroughly clean the glassware and sample cells between tests. After washing, rinse with 1:1 hydrochloric acid solution, then rinse several times with deionized water.

Maintaining the System

- With proper care and storage, the Mercury Absorber Column may be used an unlimited number of times.
- Replace the Mercury Scrubber in the air trap housing at least once for every reagent set used.
- Moisture build up on the Gas Washing Bottle side of the Acro 50 Vent Filter will reduce the purging air flow rate. If this occurs replace the filter or dry it in an oven at 110 °C.

Summary of Method

The sample is digested to convert all forms of mercury in the sample to mercuric (Hg^{2+}) ions. The mercuric ions in the digested sample are converted to mercury vapor in a semi-closed system. The vapor is carried into a chemically activated absorber column by ambient air where the mercury vapor is converted to mercuric chloride.

The mercuric chloride is eluted off the column and a sensitive indicator is added. The instrument is zeroed using the absorbance peak of the unreacted indicator. A complexing agent is added to break the mercury:indicator complex. The increase in unreacted indicator causes an increase in absorbance s proportional to the amount of mercury in the original sample. Test results are measured at 412 nm.

Safety

Wear personal protective equipment such as safety glasses with side shields, or a face shield to protect your eyes. Use other protective equipment as necessary (such as a fume hood) to avoid chemical exposure. Perform all steps exactly as prescribed in the procedure.

Pollution Prevention and Waste Management

Proper management and disposal of waste is the responsibility of the waste generator. Hach Company provides waste disposal information as a guideline only. It is up to the generator to arrange for proper disposal and comply with applicable local, state, and federal regulations governing waste disposal. Hach Company makes no guarantees or warranties, express or implied, for the waste disposal information represented in this procedure.

1. Dispose of the solution in the Gas Washing Bottle by neutralizing the solution to a pH of 6–9 and flushing to the sanitary sewer with water for several minutes.
2. The mercury contained in one liter of sample is concentrated by a factor of 100 by the Mercury Absorber Column. Mercury analysis within the range of the test may produce a solution in the sample cell that is above the RCRA Toxicity Characteristic limit of 0.20 mg/L Hg. The sample cell will contain 0.25 mg/L mercury if the original sample was at 2.5 µg/L mercury (the upper limit of the test range). Dispose of the solution in the sample cell as a hazardous waste if the test result was over 2 µg/L mercury in the original sample. Otherwise, pour the solution into the sanitary sewer and flush with water for several minutes.
3. The mercury scrubber will capture mercury vapor if the Mercury Absorber Column is not properly activated using HgEx Reagent B and HgEx Reagent C. In addition, mercury is also captured if the capacity of the Absorber Column is exceeded. If the Mercury Scrubber has captured mercury vapor, it must be disposed of according to applicable regulations.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Cold Vapor Mercury Reagent Set (25 tests)			26583-00
Includes:			
HgEx™ Reagent A, Stannous Sulfate Solution, 20-mL ampules	1	25/pkg	26588-25
HgEx™ Reagent B, Sulfuric Acid Solution	19 mL	500 mL	26589-49
HgEx™ Reagent C, Sodium Hypochlorite Solution	2 mL	55 mL	26590-59
HgEx™ Reagent 3, Alkaline Reagent Powder Pillows	1 pillow	25/pkg	26584-48
HgEx™ Reagent 4, Indicator Powder Pillows	1 pillow	25/pkg	26585-48
HgEx™ Reagent 5, Sodium Hydroxide Solution	8 drops	10 mL SCDB	26586-36
HgEx™ Reagent 6, Complexing Reagent Powder Pillows	1 pillow	25/pkg	26587-48
Mercury Scrubber	2/reagent set	2/pkg	26558-00

Required Digestion Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Hydroxylamine Hydrochloride	varies	113 g	246-14
Nitric Acid, ACS	25 mL	500 mL	152-49
Potassium Permanganate, ACS	varies	454 g	168-01H
Potassium Persulfate, ACS	4.0 g	454 g	26175-01
Sulfuric Acid, ACS, concentrated	50 mL	4 kg	979-09

Required Apparatus

Cold Vapor Mercury Apparatus Set			26744-00
Acro 50 Vent Filter	1	18/pkg	26833-18
Air Trap Holder Assembly	1	each	26639-00
Ampule Breaker	1	each	25640-00
Breaker/Capper Tool for Mercury Scrubber	1	each	26640-00
C-flex Tubing, 0.25-inch ID, white	4 ft	25 ft	23273-67
Clamp for Mercury Absorber Column	1	each	26562-00
Clamp Holder	2	each	326-00
Cylinder, graduated, 50-mL	1	each	508-41
Distilling Receiver, 10-mL	1	each	26554-38
Flask, Erlenmeyer, 100-mL	1	each	26553-42
Funnel, micro	1	each	25843-35
Gas Washing Bottle, 1200-mL	1	each	26622-00
Glass Elbow, 90-degree, with hose adapter	1	each	26552-00
Mercury Absorber Column	1	each	26555-10
Pipet, TenSette®, 0.1 to 1.0 mL		each	19700-01
Pipet, TenSette®, 1.0 to 10.0 mL		each	19700-10
Pipet Tips, for 19700-01 TenSette® Pipet		50/pkg	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet		50/pkg	21997-96
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Support Ring for Gas Washing Bottle	1	each	26563-00
Stopper, for Distilling Receiver	1	each	26559-00
Stopper, for Gas Washing Bottle	1	each	26623-00
Support, Base and Rod	1	each	329-00
Tubing Quick Disconnect, HDPE	1	12/pkg	14810-00
Vacuum Pump, with fittings, 115 VAC		each	26557-00
Vacuum Pump, with fittings, 230 VAC		each	26557-02

Required Digestion Apparatus

Flask, Erlenmeyer, 2000-mL	1	each	24894-54
Hot Plate/Stirrer, 120 VAC	1	each	23442-00
Hot Plate/Stirrer, 240 VAC	1	each	23442-02
Spoon, measuring, 0.5-g	1	each	907-00
Stir Bar	1	each	20953-55
Thermometer, -20 to 110 °C	1	each	566-01
Watch Glass, Pyrex, 65-mm	1	each	578-67

Required Standards

Mercury Standard Solution, 1000-mg/L Hg (NIST)		100 mL	14195-42
Water, deionized		4 L	272-56

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WORLD HEADQUARTERS
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FAX: (970) 669-2932

Metolachlor

Immunoassay Method*

Scope and Application: For water

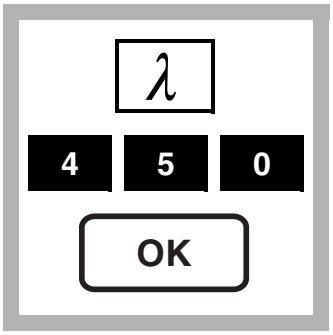
* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

This method analyzes for Metolachlor in water. Sample calibrators and reagents are added to cuvettes coated with Metolachlor-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

Tips and Techniques

- **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis. A 1-cm square cell holder is required for this procedure.
- **Timing is critical**; follow instructions carefully.
- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack on page 5.* Cuvettes can be mixed individually, but test results may not be consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the spectrophotometer.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
- Hach Company recommends wearing protective nitrile gloves for this procedure.

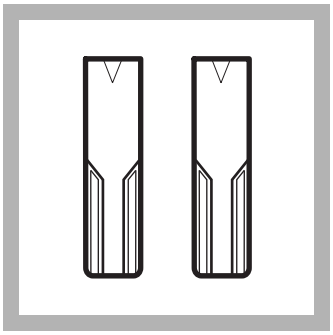
Immunoassay Procedure for Water



1. Touch

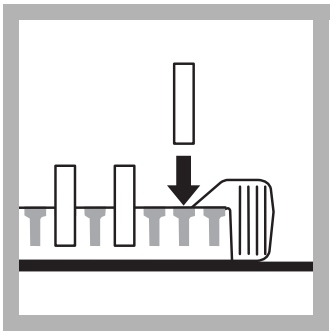
Single Wavelength

then touch the λ button. Type in 450 nm and touch **OK.**

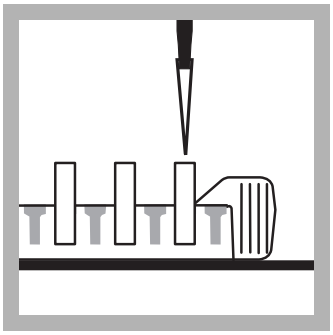


2. Label an Antibody Cuvette for each calibrator and each sample to be tested.

Note: *As many as 20 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.*

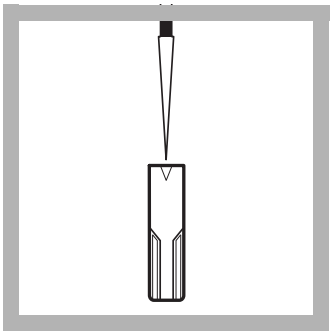


3. Place the cuvettes into the rack snugly.



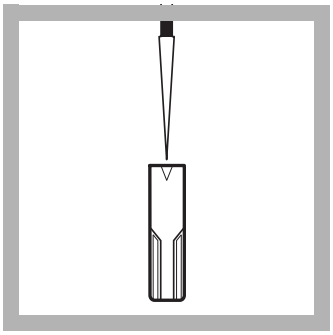
4. Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

Note: *Use a new pipette tip for each calibrator.*

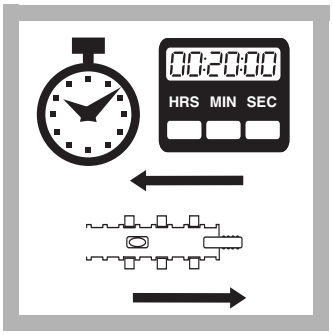


5. Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

Note: *Use a new pipette tip for each sample.*



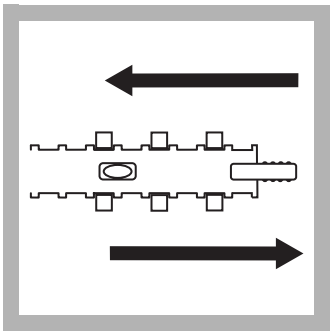
6. Immediately pipet 0.5 mL of Metolachlor Enzyme Conjugate into each cuvette.



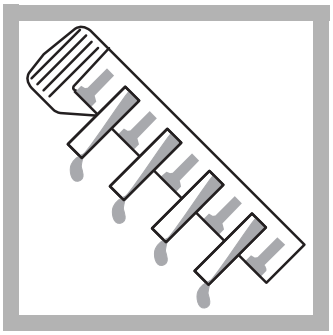
7. Touch the timer icon. Enter 20 minutes and touch **OK.**

A 20-minute reaction time will begin.

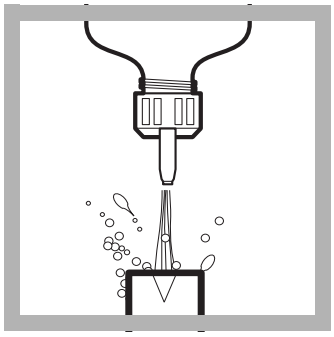
Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack on page 5*.



8. After 10 minutes mix the contents of the rack for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack on page 5*.



9. At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

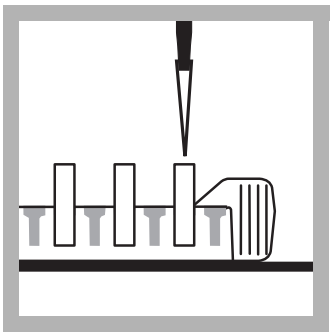


10. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: *Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.*

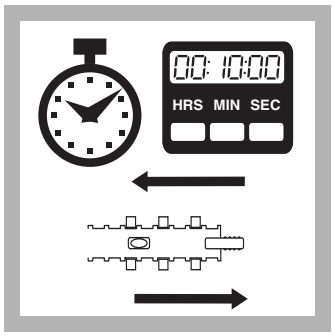
Color Development

Note: *Timing is critical; follow instructions carefully.*



11. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: *Use a new pipette tip for each cuvette.*

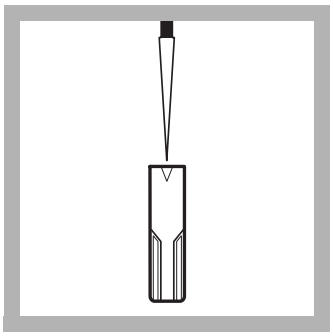


12. Touch the timer icon. Enter 10 minutes and touch **OK.**

A reaction period will begin. Mix, using the instructions on page 5 in *Using the 1-cm MicroCuvette Rack*.

13. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Note: *Solutions will turn blue in some or all of the cuvettes.*



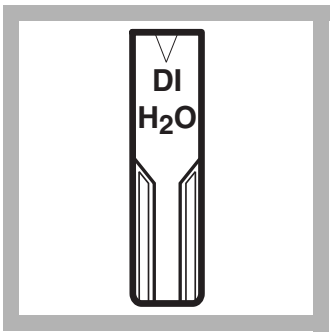
14. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in *step 11*.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

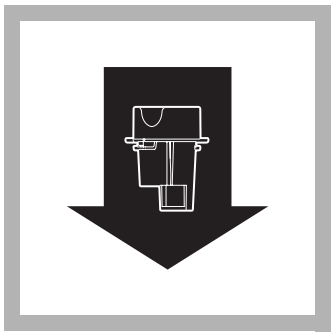
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

Note: *The same pipette tip can be used repeatedly for this step.*

Measuring the Color



15. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

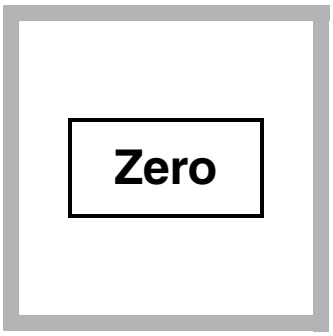


16. Install the 1-cm square cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Place the filled zeroing cuvette into the cell holder—arrow pointing towards the left side of the instrument.

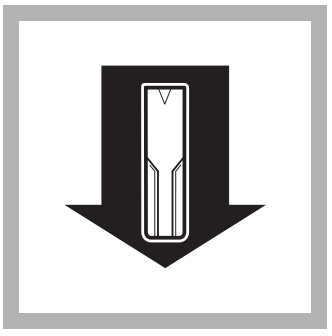
Orient the arrow in the same direction for all cuvettes.



17. Touch **Zero**.

The display will show:

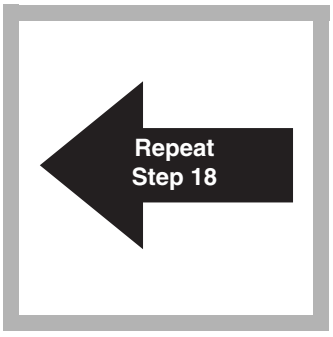
0.000 Abs



18. Place the first calibrator into the celladapter.

Touch **Read**.

The display will give an absorbance reading. Record the results for each calibrator and sample.



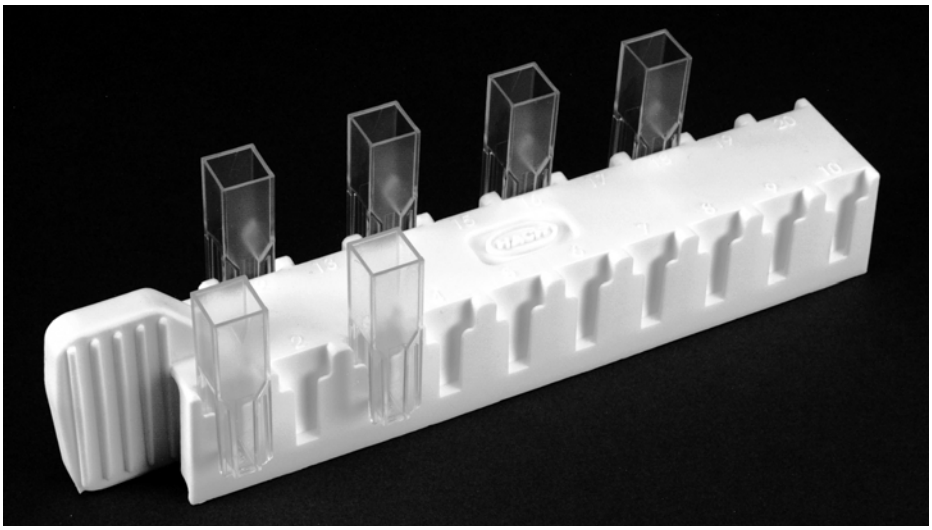
19. Repeat *step 18* for all remaining calibrators and samples.

See *Interpreting and Reporting Results* for help with interpretation of results.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 1*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 1 The 1-cm MicroCuvette Rack



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of Metolachlor and the reading. In other words, the higher the reading, the lower the concentration of Metolachlor.

If the sample reading is...	the sample Metolachlor Concentration is...
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example

Readings:

0.5 ppb Metolachlor Calibrator: **0.475 Abs**
2.0 ppb Metolachlor Calibrator: **0.245 Abs**

Sample #1: **0.140 Abs**

Sample #2: **0.300 Abs**

Sample #3: **0.550 Abs**

Interpretation

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Metolachlor is greater than both 0.5 ppb and 2.0 ppb Metolachlor.

Sample #2 — Sample reading is between the readings for the 0.5 ppb and 2.0 ppb Metolachlor calibrators. Therefore the sample concentration of Metolachlor is between 0.5 ppb and 2.0 ppb.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Metolachlor is less than both 2.0 ppb and 0.5 ppb.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The Metolachlor immunoassay test cannot differentiate between certain herbicides and metabolites, but it detects their presence to differing degrees. The following table shows the required concentration for selected chemicals.

Compound	Concentration to give a positive response of 0.5 ppb Metolachlor	Concentration to give a positive response of 2.0 ppb Metolachlor
Acetochlor	74 ppm	398 ppm
Butachlor	84 ppb	550 ppb
2 Chloro-2',6'-Diethylacetaniline	8 ppm	60 ppm
2,6-Diethylaniline	61 ppm	313 ppm
Propachlor	60 ppb	295 ppb

Sample Collection and Storage

The following compounds are not detectable at 10,000 ppb:		
Atrazine	Carbofuran	Carbendazim
Aldicarb	2, 4-D	
Diazoton	Chlorpyirfos	

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Metolachlor-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Metolachlor from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Metolachlor compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Metolachlor and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Metolachlor in the sample. The resulting color is then compared with a calibrator to determine whether the Metolachlor concentration in the sample is greater or less than the threshold levels.

Test results are measured at 450 nm.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, Metolachlor*	20 cuvettes	28135-00

Required Apparatus

Adapter, 1-cm square cell	each	59459-00
Caps, flip spout	2/pkg	25818-02
Cell holder, 1-cm square	each	59065-00
Marker, laboratory	each	20920-00
Rack, for 1-cm Micro Cuvettes	each	48799-00
Wipes, disposable	box	20970-00
TenSette®, Pipet, 0.1–1.0 mL	each	19700-01
Tips, for pipettor 19700-01	1000/pkg	21856-28

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.

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FAX: (970) 669-2932

3/04 1ed

MET_None_Other_IMM_Eng_Ody.fm

Molybdenum, Molybdate

Method 8036 **Mercaptoacetic Acid Method***

Powder Pillows or AccuVac® Ampuls **HR (0.3 to 40.0 mg/L)**

Scope and Application: For water and wastewater.

* Adapted from *Analytical Chemistry, 25(9) 1363 (1953)*

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Filter turbid samples using filter paper (Cat. No. 1894-57) and a funnel (Cat. No. 1083-67).
- After all reagents have been added, the presence of molybdenum will cause a yellow color to form.

Powder Pillows — Method 8036

1. Touch

Hach Programs.

Select program

320 Molybdenum HR.

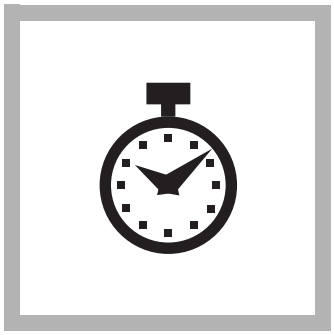
Touch **Start**.

2. Fill a sample cell with 10-mL of sample.

3. Add the contents of one MolyVer® 1 Reagent Powder Pillow. Swirl to mix.

4. Add the contents of one MolyVer 2 Reagent Powder Pillow. Swirl to mix.

5. Add the contents of one MolyVer 3 Reagent Powder Pillow. Swirl to mix (the prepared sample).



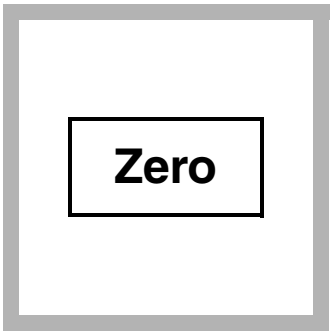
6. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

7. When the timer beeps, fill a second cell with 10 mL of the original sample (the blank).

8. Insert the blank into the cell holder.

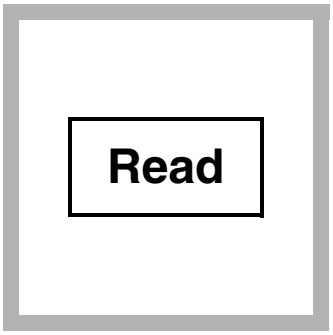


9. Touch **Zero**.

The display will show:

0.0 mg/L Mo^{6+}

10. Place the prepared sample into the cell holder.



11. Touch **Read**.

Results will appear in mg/L Mo^{6+}.

AccuVac Ampul — Method 8036

1. Touch

Hach Programs.

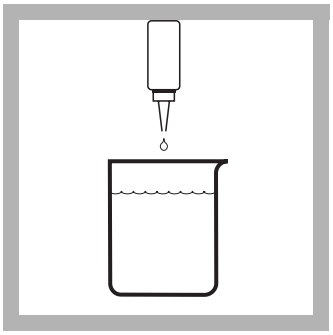
Select program

322 Molybdenum HR AV.

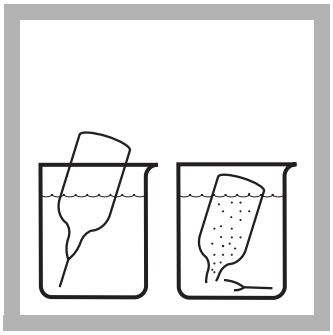
Touch **Start**.

2. Fill a sample cell with 10 mL of sample (the blank).

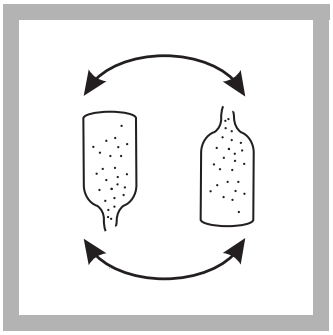
Collect 40 mL of sample in a 50-mL beaker.



3. Add four drops of 0.4 M CDTA Standard Solution to the sample in the beaker. Swirl to mix.

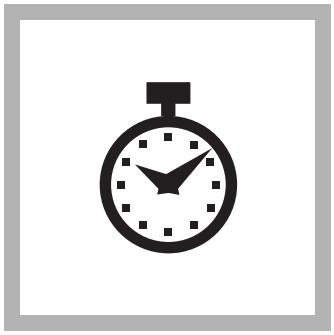


4. Fill a MolyVer 6 AccuVac® Ampul with the treated sample.



5. Invert the ampule several times to mix (the prepared sample).

Undissolved reagent will not affect test results.

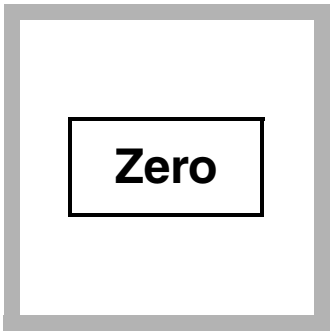


6. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

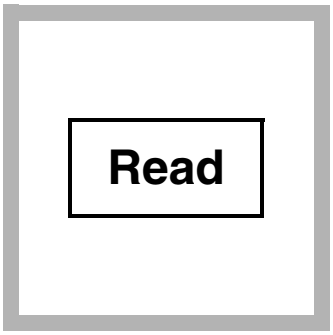
7. When the timer beeps, insert the blank into the cell holder.



8. Touch **Zero**.

The display will show:

0.0 mg/L Mo^{6+}



9. Place the prepared sample into the cell holder.

10. Touch **Read**.

Results will appear in mg/L Mo^{6+}.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 50 mg/L
Chromium	Greater than 1000 mg/L
Copper	Samples containing 10 mg/L copper or more will exhibit an increasing positive interference upon standing. Read these samples as soon as possible after the five minute reaction period is complete.
Iron	Greater than 50 mg/L
Nickel	Greater than 50 mg/L
Nitrite	Interference from up to 2000 mg/L as NO_2^- can be eliminated by adding one Sulfamic Acid Powder Pillow (Cat. No. 1055-99) to the sample.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see *Section 3.3 Interferences* on page *41*.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Adjust the pH to 2 or less with nitric acid (about 2 mL/L). Preserved samples can be stored up to 6 months at room temperature. Adjust the pH to 7 with 5.0 N Sodium Hydroxide before analysis. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a bottle of Molybdenum Standard Solution, 1000-mg/L Mo^{6+}.

5. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 30 mL of sample. Use the TenSette® Pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of standard, respectively, to each sample and mix thoroughly.

Note: *For AccuVac Ampuls, fill three Mixing Cylinders (Cat. No. 1896-42) with 60-mL of sample and spike with 0.4 mL, 0.8 mL, and 1.2 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL Beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

6. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

To assure the accuracy of the test, use a Molybdenum Standard Solution, 10.0-mg/L Mo^{6+}. Follow the procedure for powder pillows or AccuVac Ampuls.

Standard Adjust

1. Use a Molybdenum Standard Solution, 10.0 mg/L Mo^{6+} to perform the molybdenum procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 10.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 10.0 mg/L Mo^{6+}

Program	95% Confidence Limits of Distribution
320	9.9–10.1 mg/L Mo^{6+}
322	9.8–10.2 mg/L Mo^{6+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
320	Entire range	0.010	0.3 mg/L Mo^{6+}
322	Entire range	0.010	0.1 mg/L Mo^{6+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

MolyVer 1 and 2 Reagents are added to buffer and condition the sample. MolyVer 3 provides the mercaptoacetic acid which reacts with molybdate molybdenum to form a yellow color proportional to the molybdenum concentration. Test results are measured at 420 nm.

Required Reagents Standards (Using Powder Pillows)

Description	Quantity Required per test	Unit	Cat. No.
Molybdenum Reagent Set, for 10-mL samples (100 tests)			26041-00
Includes:			
MolyVer® 1 Molybdenum Reagent Powder Pillows	1 pillow	100/pkg	26042-99
MolyVer® 2 Molybdenum Reagent Powder Pillows	1 pillow	100/pkg	26043-99
MolyVer® 3 Molybdenum Reagent Powder Pillows	1 pillow	100/pkg	26044-99

Required Reagents (Using AccuVac Ampuls)

CDTA Solution, 0.4 M	4 drops	15 mL SCDB	26154-36
MolyVer® 6 Reagent AccuVac® Ampuls	1 ampul	25/pkg	25220-25

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Molybdenum Standard Solution, 10-mg/L as MoO_4^{2-}		100 mL	14187-42
Molybdate Standard Solution, 1000-mg/L as MoO_4^{2-}		100 mL	25962-42
Molybdenum Standard Solution, 1000-mg/L as Mo^{6+}		100 mL	14186-42
Water, deionized		4 liters	272-56

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Molybdenum, Molybdate

Method 8169

Ternary Complex Method

Powder Pillows

LR (0.02 to 3.00 mg/L)

Scope and Application: For boiler and cooling tower waters

Tips and Techniques

- Analyze samples immediately after collection.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Filter turbid samples using filter paper (Cat. No. 1894-57) and a funnel (Cat. No. 1083-67).

Powder Pillows — Method 8169

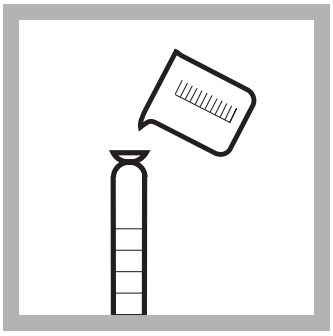
1. Touch

Hach Programs.

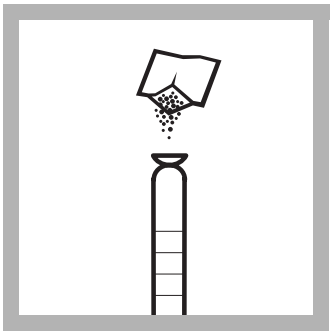
Select program

315 Molybdenum LR.

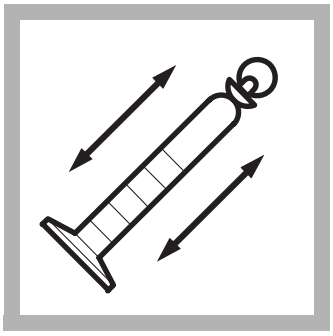
Touch **Start**.



2. Fill a 25-mL graduated mixing cylinder with 20 mL of sample.



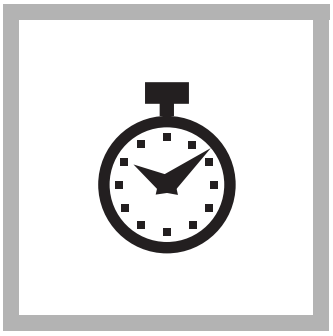
3. Add the contents of one Molybdenum 1 Reagent Powder Pillow to the graduated cylinder.



4. Stopper the cylinder and shake to dissolve the reagent (this is the prepared sample).

5. Pour 10-mL of the prepared sample into a round sample cell.

6. Add 0.5 mL of Molybdenum 2 Reagent to the sample cell. Swirl to mix. This is the developed sample.



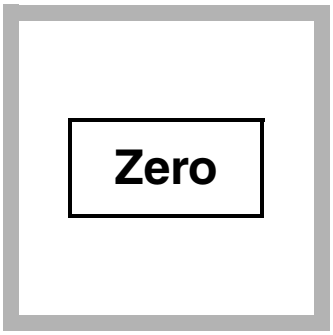
7. Touch the timer icon.

Touch **OK**.

A two-minute reaction period will begin.

8. When the timer beeps, fill a second sample cell with 10 mL of the remaining prepared sample (this is the blank).

9. Wipe the blank and place it into the cell holder.

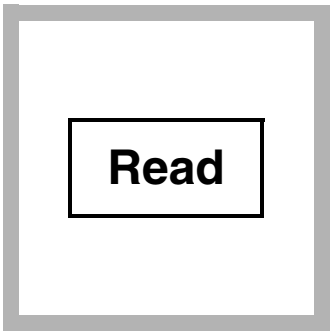


10. Touch **Zero**.

The display will show:

0.00 mg/L Mo^{6+}

11. Wipe the developed sample and place it into the cell holder.



12. Touch **Read**.

Results will appear in mg/L Mo^{6+}.

Interferences

Interferences studies were conducted by preparing a molybdenum standard solution (2-mg/L Mo^{6+}) as well as a solution of the potential interfering ion. When the standard solution concentration changed by ±5% with a given ion concentration, the ion was considered an interference. The tables below list the details of these studies.

Table 1 Substances that Cause a Negative Interference

Interfering Substance	Interference Levels and Treatments
Alum	Greater than 7 mg/L
Aluminum	Greater than 2 mg/L
AMP (Phosphonate)	Greater than 15 mg/L
Bicarbonate	Greater than 5650 mg/L
Bisulfate	Greater than 3300 mg/L
Borate	Greater than 5250 mg/L
Chloride	Greater than 1400 mg/L
Chromium	Greater than 4.5 mg/L*
Copper	Greater than 98 mg/L
Diethanoldith-iocarbamate	Greater than 32 mg/L
EDTA	Greater than 1500 mg/L
Ethylene Glycol	Greater than 2% (by volume)
Iron	Greater than 200 mg/L
Lignin Sulfonate	Greater than 105 mg/L
Nitrite	Greater than 350 mg/L
Orthophosphate	Greater than 4500 mg/L
Phosphonohydroxyacetic Acid	Greater than 32 mg/L
HEDP (Phosphonate)	The presence of the phosphonate HEDP at concentrations up to 30 mg/L will increase the apparent molybdenum concentration reading by approximately 10% (positive interference). Multiply the value obtained in *step 12* by 0.9 to obtain the actual Mo^{6+} concentration.

Table 1 Substances that Cause a Negative Interference (continued)

Interfering Substance	Interference Levels and Treatments
Sulfite	Greater than 6500 mg/L

* Read the molybdenum concentration immediately after the beep of the 2-minute reaction period.

Table 2 Substances that Cause a Positive Interference

Interfering Substance	Interference Levels and Treatments
Benzotriazole	Greater than 210 mg/L
Carbonate	Greater than 1325 mg/L
Morpholine	Greater than 6 mg/L
Phosphonate HEDP	Positive interference of about 10% up to 30 mg/L. As the concentration increases above 30 mg/L, a decrease in the molybdenum concentration reading occurs (negative interference).
Silica	Greater than 600 mg/L

Table 3 Non-Interfering Substances

Substance	Highest Concentration Tested
Bisulfite	9600 mg/L
Calcium	720 mg/L
Chlorine	7.5 mg/L
Magnesium	8000 mg/L
Manganese	1600 mg/L
Nickel	250 mg/L
PBTC (phosphonate)	500 mg/L
Sulfate	12,800 mg/L
Zinc	400 mg/L

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagent and require sample pretreatment. Adjust the sample pH to between 3–5 by adding, dropwise, an appropriate amount of acid or base such as 1.0 N Sulfuric Acid Standard Solution (Cat. No. 1270-32), or 1.0 N Sodium Hydroxide Standard Solution (Cat. No. 1045-32). If significant volumes of acid or base are used, a volume correction should be made by dividing the total volume (sample + acid + base) by the original volume and multiplying the test result by this factor.

After a number of samples have been analyzed, the sample cells may exhibit a slight blueish discoloration. Rinse the cells with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) to eliminate this build-up.

Sample Collection, Storage and Preservation

Collect samples in glass or plastic bottles. Analyze samples immediately.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Molybdenum Voluette® Ampule Standard, 500-mg/L Mo^{6+}.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-42) with 100 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze 20 mL of each standard addition sample as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, a graph of the best-fit line through the standard additions data points will appear, accounting for matrix interferences. Touch **Ideal Line** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

1. Using Class A glassware, prepare a 2.00-mg/L molybdenum standard solution by pipetting 10.00 mL of Molybdenum Standard Solution, 10.00-mg/L, into a 50-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the molybdenum procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 2.00-mg/L Mo standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 2.00 mg/L Mo^{6+}

Program	95% Confidence Limits of Distribution
315	1.93–2.07 mg/L Mo^{6+}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.02 mg/L Mo^{6+}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The ternary complex method for molybdenum determination is a method in which molybdate molybdenum reacts with an indicator and sensitizing agent to give a stable blue complex. Test results are measured at 610 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Molybdenum Reagent Set for 20-mL sample (100 tests)			24494-00
Includes:			
(1) Molybdenum 1 Reagent (LR) Molybdate Powder Pillows	1 pillow	100/pkg	23524-49
(1) Molybdenum 2 Reagent Solution	0.5 mL	50 mL MDB	23525-12

Required Apparatus

Cylinder, graduated mixing, 25-mL	1	each	1896-40
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Molybdenum Standard Solution, 10-mg/L Mo^{6+}	100 mL	14187-42
Molybdenum Standard Solution, 10-mL Voluette® ampule, 500-mg/L Mo^{6+}	16/pkg	14265-10
Water, deionized	4 liters	272-56

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

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★Method 8037 Heptoxime Method*

Powder Pillows (0.02 to 1.80 mg/L Ni)

Scope and Application: For water, wastewater, and seawater; USEPA accepted for reporting wastewater analyses (digestion required, see *Section 2* for digestion procedure)**

* Adapted from *Chemie Analytique*, 36 43 (1954)
** Procedure is equivalent to Standard Method 3500-Ni D for wastewater

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Make the cotton plug pea-size. A larger plug will restrict the flow; a smaller plug may become dislodged from the delivery tube of the funnel.
- Chloroform (D022) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Water saturated with chloroform, chloroform solutions, and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent wastes. See *Section 4* on page *55* for more information on proper disposal of these materials.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows Method 8037

1. Touch

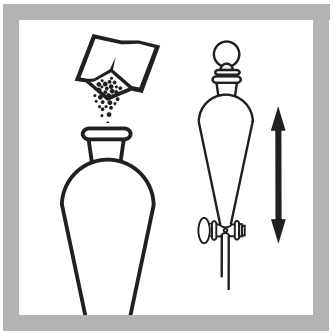
Hach Programs.

Select program

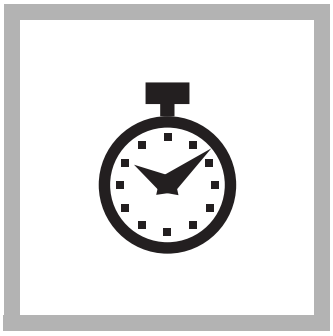
335 Nickel, Heptoxime.

Touch **Start**.

2. Measure 300 mL of sample in a 500-mL graduated cylinder. Pour into a 500-mL separatory funnel.



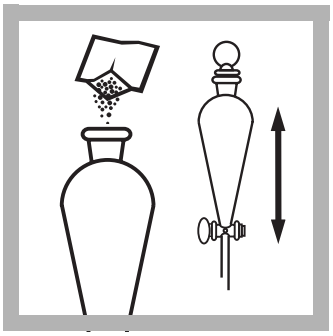
3. Add the contents of one Nickel 1 Reagent Powder Pillow to the funnel. Stopper and invert to mix.



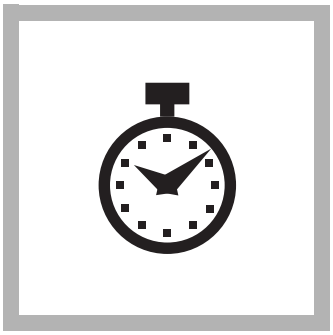
4. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.



5. When the timer beeps, add the contents of one Nickel 2 Reagent Powder Pillow to the funnel. Stopper and invert to mix.

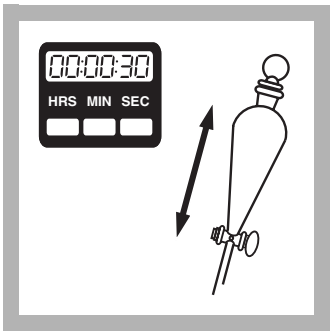


6. Touch the timer icon.

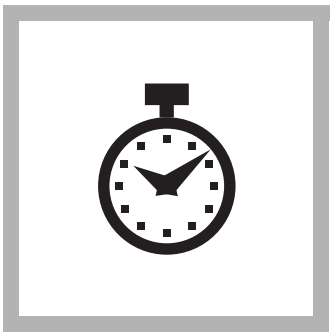
Touch **OK**.

A second five-minute reaction period will begin.

7. When the timer beeps, add 10 mL of chloroform. Stopper and invert gently. With the funnel inverted and the tip pointed away from people, open the stopcock to vent.



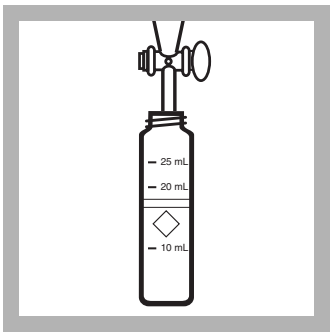
8. Close the stopcock and invert for 30 seconds.



9. Touch the timer icon.

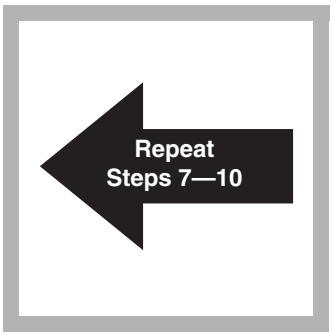
Touch **OK**.

A third five-minute reaction period will begin. Invert the funnel several times over the five minute period.



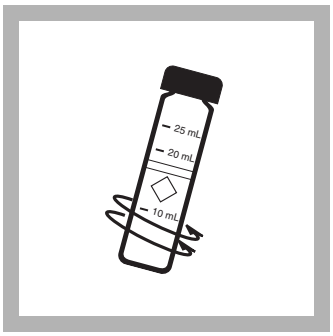
10. When the timer beeps, wait for the layers to separate. Insert a pea-sized cotton plug into the delivery tube of the funnel. Remove the stopper and drain the chloroform layer (bottom layer) into a sample cell. (This is the prepared sample.)

Stopper the funnel.

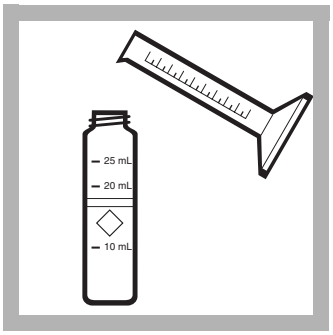


11. Repeat *steps 7* through *10* two additional times with 10-mL volumes of chloroform. The five-minute reaction period is not necessary.

Stopper the funnel and invert to mix. Wait for the layers to separate, and continue.



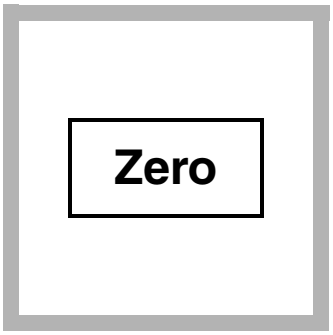
12. Cap the sample cell and invert to mix the extracts. The final volume will be about 25 mL due to the slight solubility of chloroform in water.



13. Fill a second cell with 25 mL of chloroform. (This is the blank.)

Cap the cell.

14. Wipe the blank and place it into the cell holder.



15. Touch **Zero**.

The display will show:

0.00 mg/L Ni

16. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L Ni.

Interferences

Cobalt, copper, and iron interferences can be overcome by adding additional Nickel 1 Reagent Powder Pillows in step 3. The tolerance limits of these interferences are shown in the following table:

Pillows of Nickel 1 Reagent	Tolerance Limit (mg/L):		
	Cobalt	Copper	Iron
1	1	10	20
2	7	16	65
3	13	22	110
4	18	28	155
5	25	35	200

A preliminary acid digestion is required to determine any suspended or precipitated nickel and to eliminate interference by organic matter. To eliminate this interference or to determine total recoverable nickel perform the USEPA approved digestion in *2.3 Digestion*.

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with Nitric Acid (Cat. No. 2540-49), about 5 mL per liter. Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the sample pH to between 3–8 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53). Do not exceed pH 8 as this may cause some loss of nickel as a precipitate. Correct the test results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Nickel Voluette® Ampule Standard, 300-mg/L Ni.
5. Prepare three sample spikes. Use the TenSette® Pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of standard, respectively, to three 300-mL samples and mix thoroughly.
6. Analyze as described in the procedure. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

1. Prepare a 10.0-mg/L nickel working standard solution by pipetting 10.0 mL of a Nickel Standard Solution, 1000-mg/L, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Prepare a 1.0-mg/L nickel standard solution by diluting 50.0 mL of the 10-mg/L working standard solution to 500 mL in a volumetric flask. Perform the heptoxime procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 1.00 mg/L Ni

Program	95% Confidence Limits of Distribution
335	0.93–1.07 mg/L Ni

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.02 mg/L Ni

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Nickel ion reacts with heptoxime to form a yellow-colored complex which is then extracted into chloroform to concentrate the color and enable a more sensitive determination. Chelating agents are added to the sample to overcome the interferences caused by cobalt, copper and iron. Readings are taken at 430 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Nickel Reagent Set (50 Tests)			22435-00
Includes:			
(3) Chloroform, ACS	30 mL	500 mL	14458-49
(2) Nickel 1 Reagent Powder Pillows	1 pillow	25/pkg	2123-68
(2) Nickel 2 Reagent Powder Pillows	1 pillow	25/pkg	2124-68

Required Apparatus

Clippers, for opening powder pillows	1	each	968-00
Cotton balls, absorbent	1	100/pkg	2572-01
Cylinder, graduated, 10-mL	1	each	508-38
Cylinder, graduated, 500-mL	1	each	508-49
Funnel, separatory, 500-mL	1	each	520-49
Ring, support, 4-inch	1	each	580-01
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Stand, support, 5" X 8" base	1	each	563-00
Stopper, hollow, Size 1	2	6/pkg	14480-00

Required Standards

Nickel Standard Solution, 1000-mg/L Ni	100 mL	14176-42
Nickel Standard Solution, 300-mg/L Ni, 10-mL Voluette® Ampules	16/pkg	14266-10
Water, deionized	4 liters	272-56

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Method 8150 **1-(2 Pyridylazo)-2-Napthol (PAN) Method***

Powder Pillows **(0.007 to 1.000 mg/L)**

Scope and Application: For water and wastewater; digestion is required for determining total nickel

* Adapted from Watanabe, H., *Talanta*, 21 295 (1974)

Tips and Techniques

- Cobalt concentration can be determined with the same sample by using Program Number 110.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows Method 8150

1. Touch

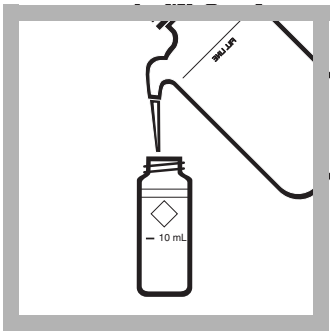
Hach Programs.

Select program

340 Nickel, PAN.

Touch **Start**.

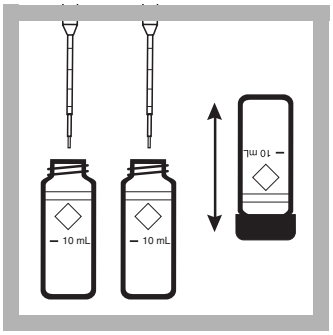
2. Fill a sample cell to the 10-mL mark with sample (this is the prepared sample).



3. Fill another sample cell to the 10-mL mark with deionized water (this is the blank).

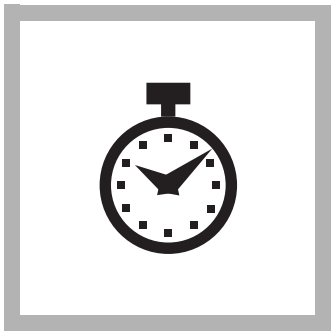
4. Add the contents of one Phthalate-Phosphate Reagent Powder Pillow to each cell. Cap. Immediately shake to dissolve.

If the sample contains iron, make sure that all the powder is dissolved before proceeding to s*tep 5.*



5. Using the plastic dropper provided, add 0.5 mL of 0.3% PAN Indicator Solution to each cell.

Cap. Invert several times to mix.



6. Touch the timer icon.

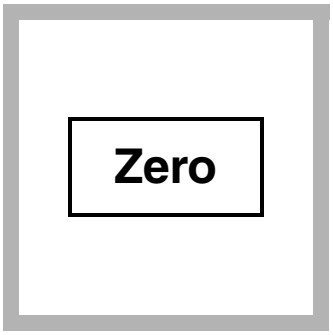
Touch **OK**.

A 15-minute reaction period will begin.

During color development, the sample solution color may vary from yellowish-orange to dark red, depending on the chemical makeup of the sample. The blank should be yellow.

7. When the timer beeps, add the contents of one EDTA Reagent Powder Pillow to each cell. Cap and shake to dissolve.

8. Wipe the blank and place it into the cell holder.



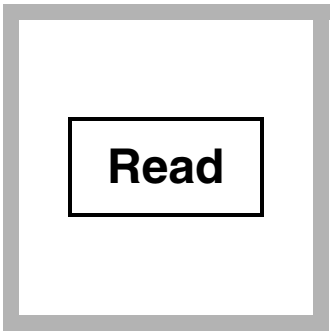
9. Touch **Zero**.

The display will show:

0.000 mg/L Ni

The instrument will zero at 560 and 620 nm.

10. Wipe the sample cell and place it into the cell holder.



11. Touch **Read**.

The instrument will read the sample at 560 and 620 nm.

Results will appear in mg/L Ni.

Interferences

Interfering Substance	Interference Levels and Treatments
Al^{3+}	32 mg/L
Ca^{2+}	1000 mg/L as ($CaCO_3$)
Cd^{2+}	20 mg/L
Cl^{-}	8000 mg/L
Chelating agents	Interfere at all levels. Use either the Digesdahl or vigorous digestion to eliminate this interference (see *Section 2*).
Cr^{3+}	20 mg/L
Cr^{6+}	40 mg/L
Cu^{2+}	15 mg/L
F^{-}	20 mg/L
Fe^{3+}	10 mg/L
Fe^{2+}	Interferes directly and must not be present.
K^{+}	500 mg/L
Mg^{2+}	400 mg/L
Mn^{2+}	25 mg/L
Mo^{6+}	60 mg/L
Na^{+}	5000 mg/L
Pb^{2+}	20 mg/L
Zn^{2+}	30 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see *Section 3.3 Interferences* on page *41*.

Sample Collection, Storage and Preservation

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with Nitric Acid (Cat. No. 2540-49), about 5 mL per liter. Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-32). If the sample is less than 10 °C, warm it to room temperature.

Do not exceed pH 8 as this may cause some loss of nickel as a precipitate. Correct test results for volume additions, see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.

4. Snap the neck off a Nickel Voluette® Ampule Standard, 50-mg/L Ni.
5. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Transfer 10 mL of each solution into a 10-mL sample cell and analyze as described in the procedure. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 5.00-mg/L Nickel stock solution by pipetting 5.00 mL of Nickel Standard Solution, 1000-mg/L as Ni, into a 1-L volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily.
2. Prepare a 0.5-mg/L Ni working solution by pipetting 10.0 mL of the 5.00-mg/L nickel stock solution into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the nickel procedure as described above.
3. To adjust the calibration curve using the reading obtained with the 0.5-mg/L working solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
4. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.500 mg/L Ni

Program	95% Confidence Limits of Distribution
340	0.492–0.508 mg/L Ni

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.007 mg/L Ni

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

After buffering the sample and masking any Fe^{3+} with pyrophosphate, the nickel is reacted with 1-(2-Pyridylazo)-2-Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal-PAN complexes except nickel and cobalt. The instrument automatically adjusts for cobalt interference by measuring the absorbance of the sample at both 560 nm and 620 nm. This method is unique because both nickel and cobalt can be determined on the same sample.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Nickel Reagent Set (100 Tests)			26516-00
Includes:			
(2) EDTA Reagent Powder Pillows	2 pillows	100/pkg	7005-99
(2) Phthalate-Phosphate Reagent Powder Pillows	2 pillows	100/pkg	26151-99
(1) PAN Indicator Solution, 0.3%	1 mL	100 mL MDB	21502-32
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Nickel Standard Solution, 1000-mg/L Ni (NIST)		100 mL	14176-42
Nickel Standard Solution, 50-mg/L Ni (NIST), 10-mL Ampules		16/pkg	25576-10

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FAX: (970) 669-2932

3/04 1ed

Dimethylglyoxime Method

UniCell™ Vials (0.10 to 6.00 mg/L)

Scope and Application: For water, wastewater, raw water, and process control

Tips and Techniques

- Undissolved or complexed nickel can only be determined after digestion with the Metal Prep Set (HCT 200).
- Adjust pH of preserved samples to between pH 3–8 before analysis.
- The outside of the vial must be clean before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- For a proof of accuracy, use the standard solution or standard addition method. (See Accuracy Check).
- See the Instrument Procedure Manual for instructions to adjust the calibration curve, if needed.
- The temperature of the water sample and the sample vial should be between 15–25 °C (59–77 °F).
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

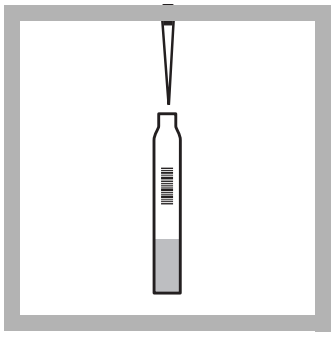
1. Touch

Hach Programs.

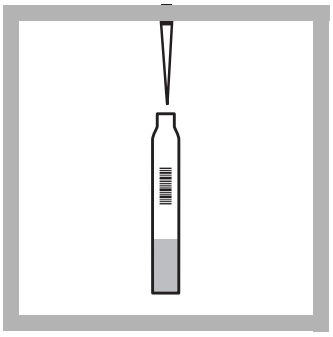
Select program

328 Nickel, HCT 167.

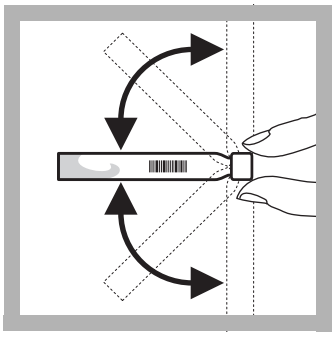
Touch **Start**.



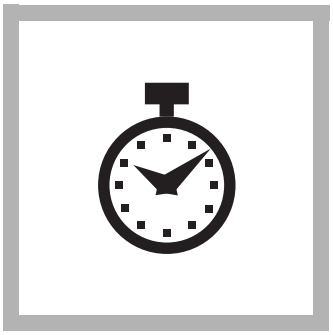
2. Pipet 4.0 mL of sample into a sample vial.



3. Pipet 0.4 mL of Dimethylglyoxime Solution A (HCT 167A) into the sample vial.



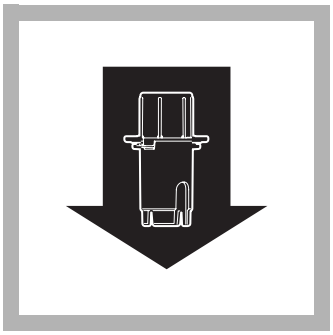
4. Cap and invert the vial several times to mix.



5. Touch the timer icon.

Touch **OK**.

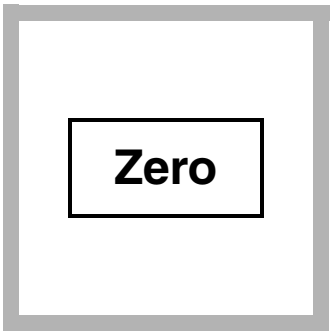
A 3-minute reaction period will begin.



6. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Wipe the zeroing vial (**white** cap) and place it into the cell adapter.

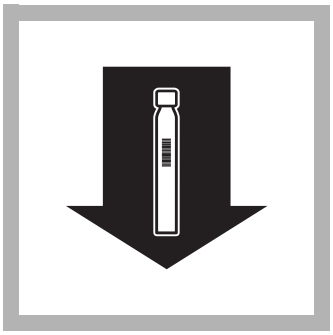


7. Touch **Zero**.

The display will show:

0.00 mg/L Free Ni
Underrange

Note: *If the sample was pretreated with the Metal Prep Set (HCT 200), display units will show:* ***mg/L Tot. Ni***



8. Wipe the sample vial and place the prepared sample into the celladapter.

Touch **Read**.

Results will appear in mg/L Free Ni.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Cl^-, Na^+, K^+, SO_4^{2-}	1000 mg/L
NH_4^+, Ca^{2+}, PO_4^{3-}, CO_3^{2-}	500 mg/L
Cr^{6+}, Zn^{2+}, F^-, NO_2^-	50 mg/L
Al^{3+}, Cr^{3+}, Cd^{2+}, Co^{2+}, Sn^{2+}, Pb^{2+}	10 mg/L
Fe^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Mg^{2+}, Hg^{2+}	5 mg/L
Ag^+	1 mg/L

Sample Collection, Storage, and Preservation

Collect samples in acid-cleaned or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to between 3–8 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 8 or nickel may precipitate.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three sample spikes. Fill three mixing cylinders with 100 mL of sample. Use a pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of 1000-mg/L Ni standard, respectively, to each sample and mix each thoroughly.
5. Transfer 4 mL of each solution into a sample vial and analyze as described in the procedure. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 2.00-mg/L Nickel stock solution by pipetting 0.20 mL of Nickel Standard Solution, 1000-mg/L as Ni, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily.
2. To adjust the calibration curve using the reading obtained with the 2.00-mg/L working solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 2.00 mg/L Free Ni

Program	95% Confidence Limits of Distribution
328	1.76–2.24 mg/L Free Ni

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
328	Entire range	0.010	0.007 mg/L Free Ni

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

In the presence of an oxidizing agent, nickel ions react with dimethylglyoxime in an alkaline solution to form an orange-brown colored complex. Measurements are taken at 463 nm.

Required Reagents

Description	Unit	Cat. No.
Nickel - Ni, UniCell™ HCT 167	23/pkg	HCT 167

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Flask, Class A, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 100-mL	each	20886-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pkg/100	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	pkg/400	27950-00
pH Paper	pkg/100	26013-00

Optional Standards

DRB 100 Digital Reactor	each	DRB 100
Metal Prep Set, HCT 200	50 digestions	HCT 200
Nickel Standard Solution, 1000-mg/L as Ni	100 mL	14176-42
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide, 5 N	1 L	2450-53

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Method 8039 **Cadmium Reduction Method**

Powder Pillows or AccuVac® Ampuls **HR (0.3 to 30.0 mg/L NO_3^-–N)**

Scope and Application: For water, wastewater, and seawater.

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- A deposit of unoxidized metal will remain after the NitraVer® 5 dissolves. The deposit will not affect results.
- This method is technique-sensitive. Shaking time and technique influence color development. For most accurate results, make successive tests on a 10-mg/L Nitrate Nitrogen Standard solution. Adjust shaking time and technique to obtain the correct result.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Rinse the sample cell immediately after use to remove all cadmium particles. Prepared samples will contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. For information on pollution prevention and waste management, refer to *Section 4* on page *55*.

Powder Pillows Method 8039

1. Touch

Hach Programs.

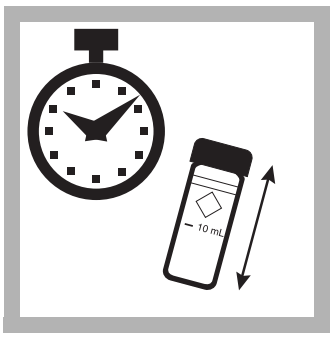
Select program

355 N, Nitrate HR.

Touch **Start**.

2. Fill a round sample cell with 10 mL of sample.

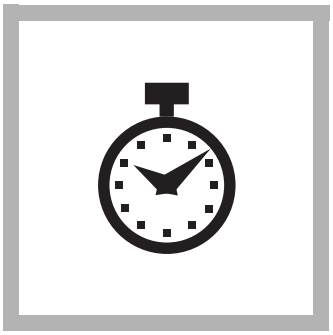
3. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow. Cap (this is the prepared sample).



4. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin. Shake the cell vigorously until the timer beeps.



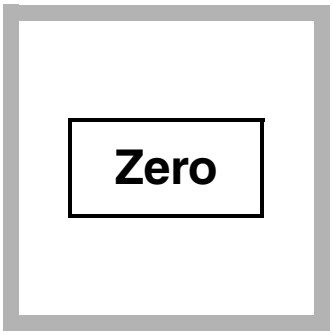
5. When the timer beeps, touch the timer icon. Touch **OK**.

A five-minute reaction period will begin.

An amber color will develop if nitrate is present.

6. When the timer beeps, fill a second round sample cell with 10 mL of sample (this is the blank).

7. Wipe the blank and place it into the cell holder.

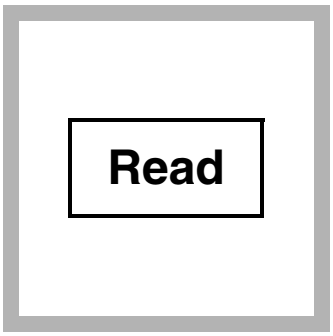


8. Touch **Zero**.

The display will show:

0.0 mg/L NO_3^-–N

9. Within one minute after the timer beeps, wipe the prepared sample and place it into the cell holder.



10. Touch **Read**.

Results will appear in mg/L NO_3^-–N.

AccuVac® Ampul — Method 8039

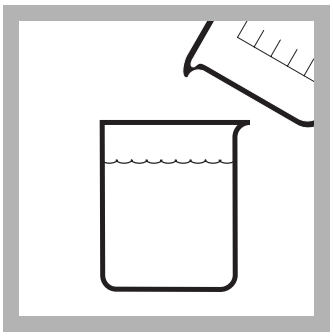
1. Touch

Hach Programs.

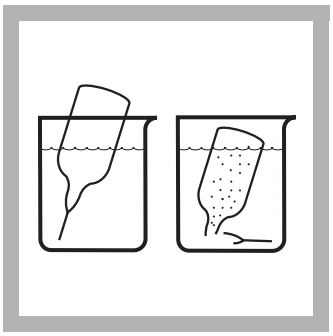
Select program

361 N, Nitrate HR AV.

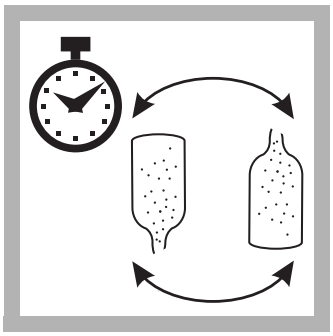
Touch **Start**.



2. Collect at least 40 mL of sample in a 50-mL beaker.



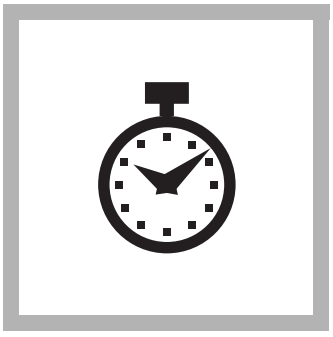
3. Fill a NitraVer 5 Nitrate AccuVac® Ampul with sample. Keep the tip immersed while the ampule fills completely. Place a stopper over the ampule tip.



4. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin. Invert the ampule 48–52 times a minute until the timer beeps.



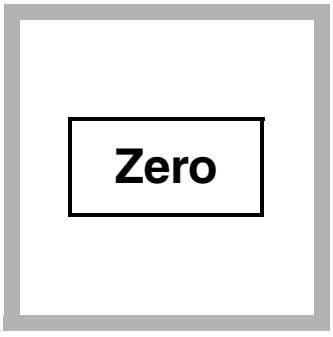
5. When the timer beeps, touch the timer icon. Touch **OK**.

A five-minute reaction period will begin.

An amber color will develop if nitrate is present.

6. When the timer beeps, fill a round sample cell with 10 mL of sample (this is the blank).

7. Wipe the blank and place it into the cell holder.

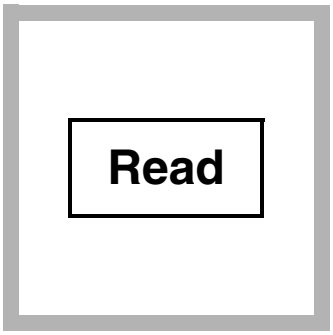


8. Touch **Zero**.

The display will show:

0.0 mg/L NO_3^-–N

9. Within one minute after the timer beeps, wipe the ampule and place into the cell holder.



10. Touch **Read**.

Results will appear in mg/L NO_3^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration.
Ferric iron	Interferes at all levels
Nitrite	Interferes at all levels Compensate for nitrite interference as follows: a) Before performing *step 3*, add 30-g/L Bromine Water (Cat. No. 2211-20) dropwise to the sample until a yellow color remains. b) Add one drop of 30-g/L Phenol Solution (Cat. No. 2112-20) to destroy the color. Proceed with step 3. Report the results as total nitrate and nitrite.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage, and Preservation

More reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (H_2SO_4) (Cat. No. 979-49) per liter and store at 4 °C.

Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53). Do not use mercury compounds as preservatives. Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Nitrate Nitrogen Voluette® Ampule Standard, 500-mg/L NO_3^-–N.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac ampules, fill three mixing cylinders (Cat. No. 1896-41) with 50 mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. To test accuracy, use a 10.0-mg/L Nitrate Nitrogen Standard Solution in place of the sample and perform the procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 10.0-mg/L Nitrate Nitrogen Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical forms). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 10 mg/L NO_3^-–N

Program	95% Confidence Limits of Distribution
355	8.0–12.0 mg/L NO_3^-–N
361	9.2–10.8 mg/L NO_3^-–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration (Program 355)	ΔConcentration (Program 361)
0 ppm	0.010	0.3 mg/L NO_3^-–N	0.5 mg/L NO_3^-–N
10 ppm	0.010	0.6 mg/L NO_3^-–N	0.7 mg/L NO_3^-–N
30 ppm	0.010	1.0 mg/L NO_3^-–N	0.8 mg/L NO_3^-–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution. Test results are measured at 500 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
NitraVer® 5 Nitrate Reagent Powder Pillows (for 10-mL sample)	1 pillow	100/pkg	21061-69
or			
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1 ampul	25/pkg	25110-25

Required Apparatus

Sample cell, 10-mL, w/cap	2	each	24276-06

Required Standards

Nitrate Nitrogen Standard Solution, 10.0-mg/L NO_3^-–N		500 mL	307-49
Nitrate Nitrogen Standard Solution, 2-mL PourRite® Ampule, 500-mg/L NO_3^-–N		20/pkg	14260-20
Water, deionized		4 liters	272-56

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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 8171 **Cadmium Reduction Method**

Powder Pillows or AccuVac® Ampuls **MR (0.1 to 10.0 mg/L NO_3^-–N)**

Scope and Application: For water, wastewater, and seawater

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- A deposit of unoxidized metal will remain after the NitraVer® 5 dissolves. The deposit will not affect results.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- This method is technique-sensitive. Shaking time and technique influence color development. For most accurate results, make successive tests on a 10.0-mg/L Nitrate Nitrogen Standard solution. Adjust shaking times to obtain the correct result.
- Rinse the sample cell immediately after use to remove all cadmium particles. Retain the used sample for proper hazardous waste disposal for cadmium.
- Prepared samples will contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. For information on pollution prevention and waste management, refer to *Section 4*.

Powder Pillows Method 8171

1. Touch

Hach Programs.

Select program

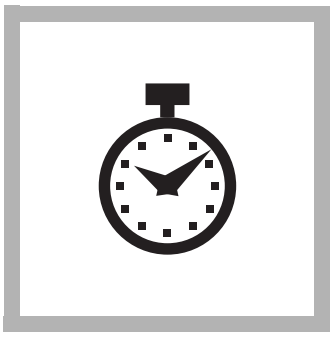
353 N, Nitrate MR.

Touch **Start**.

2. Fill a round sample cell with 10 mL of sample.

3. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow (this is the prepared sample).

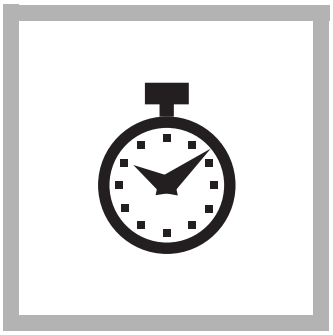
Cap.



4. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin. Shake the cell vigorously until the timer beeps.



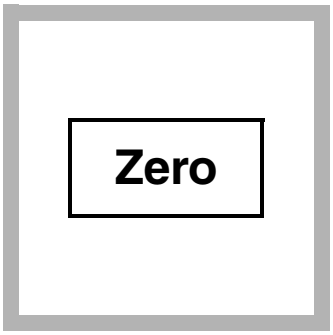
5. When the timer beeps, touch the timer icon. Touch **OK**.

A five-minute reaction period will begin.

An amber color will develop if nitrate is present.

6. When the timer beeps, fill a second round sample cell with 10 mL of sample (this is the blank).

7. Place the blank into the cell holder.

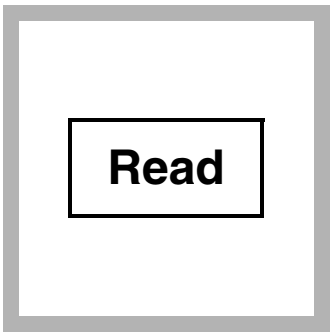


8. Touch **Zero**.

The display will show:

0.0 mg/L NO_3^-–N

9. Within two minutes after the timer beeps, place the prepared sample into the cell holder.



10. Touch **Read**.

Results will appear in mg/L NO_3^-–N.

AccuVac® Ampul — Method 8171

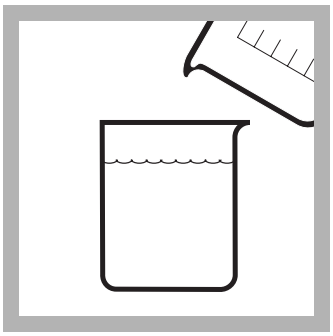
1. Touch

Hach Programs.

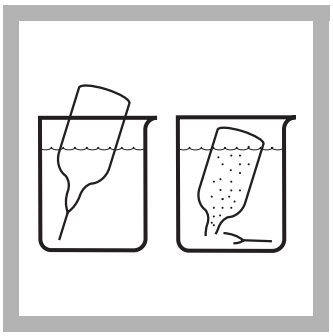
Select program

359 N, Nitrate MR AV.

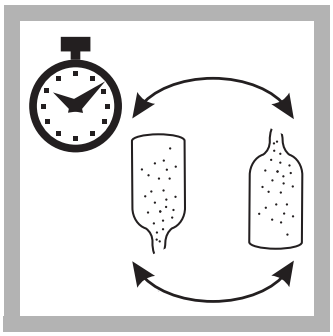
Touch **Start**.



2. Collect at least 40 mL of sample in a 50-mL beaker.



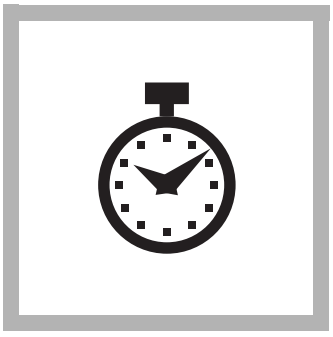
3. Fill a NitraVer 5 Nitrate AccuVac® Ampul with sample. Keep the tip immersed while the ampule fills completely. Place a stopper over the ampule tip.



4. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin. Invert the ampule 48–52 times until the timer beeps.



5. When the timer beeps, touch the timer icon. Touch **OK**.

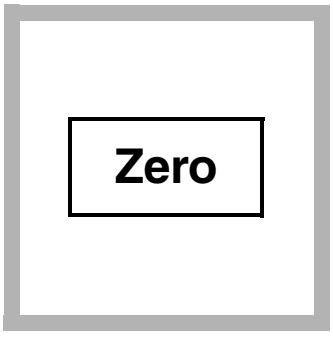
A five-minute reaction period will begin.

An amber color will develop if nitrate is present.

6. When the timer beeps, fill a round sample cell with 10 mL of sample (this is the blank).

Cap.

7. Wipe the blank and place it into the cell holder.

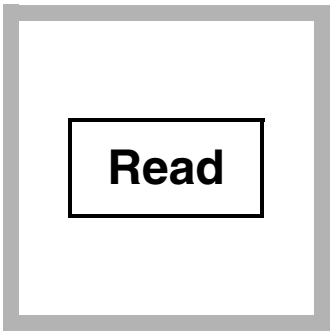


8. Touch **Zero**.

The display will show:

0.0 mg/L NO_3^-–N

9. Within two minutes after the timer beeps, wipe the ampule and place it into the cell holder.



10. Touch **Read**.

Results will appear in mg/L NO_3^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration.
Ferric iron	Interferes at all levels
Nitrite	Interferes at all levels Compensate for nitrite interference as follows: a) Add 30-g/L Bromine Water (Cat. No. 2211-20) dropwise to the sample in step 3 until a yellow color remains. b) Add one drop of 30-g/L Phenol Solution (Cat. No. 2112-20) to destroy the color. Proceed with step 4. Report the results as total nitrate and nitrite.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (H_2SO_4) (Cat. No. 979-49) per liter and store at 4 °C.

Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53). Do not use mercury compounds as preservatives. Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Nitrate Nitrogen Voluette® Ampule Standard, 100-mg/L NO_3^-–N.
5. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac ampules, fill three Mixing Cylinders (Cat. No. 1896-41) with 50 mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL Beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. To test accuracy, use a 10.0-mg/L Nitrate Nitrogen Standard Solution in place of the sample and perform the procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 10.0-mg/L Nitrate Nitrogen Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 10 mg/L NO_3^-–N

Program	95% Confidence Limits of Distribution
353	8.0–12.0 mg/L NO_3^-–N
359	9.2–10.8 mg/L NO_3^-–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
353	Entire range	0.010	0.1 mg/L NO_3^-–N
359	Entire range	0.010	0.1 mg/L NO_3^-–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution. Test results are measured at 430 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
NitraVer® 5 Nitrate Reagent Powder Pillows (for 10 mL sample)	1 pillow	100/pkg	21061-69
or			
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1 ampul	25/pkg	25110-25

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Beaker, 50-mL	1	each	500-41H

Required Standards

Nitrate Nitrogen Standard Solution, 1.00-mg/L NO_3^-–N	500 mL	2046-49
Nitrate Nitrogen Standard Solution, 10.0-mg/L NO_3^-–N	500mL	307-49
Nitrate Nitrogen Standard Solution, 100-mg/L NO_3^-–N	500 mL	1947-49
Water, deionized	4 liters	272-56

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Method 8192 **Cadmium Reduction Method**

Powder Pillows **LR (0.01 to 0.50 mg/L NO_3^-–N)**

Scope and Application: For water, wastewater, and seawater

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- A deposit of unoxidized metal will remain after the NitraVer® 6 dissolves. The deposit will not affect results.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Rinse the sample cell and mixing cylinder immediately after use to remove all cadmium particles.
- Properly dispose of the used sample. Prepared samples contain cadmium and must be disposed of according to Federal, State, and local hazardous waste regulations. For information on pollution prevention and waste management, see *Section 4* on page *55*.
- Shaking time and technique influence color development. Analyze a standard solution several times and adjust the shaking time to obtain the correct result. Use this time for analyzing samples

Powder Pillows Method 8192

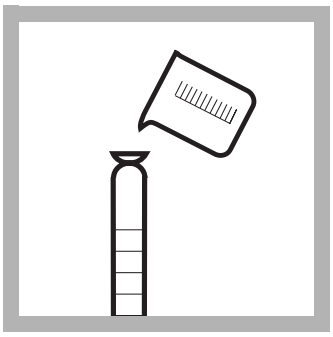
1. Touch

Hach Programs.

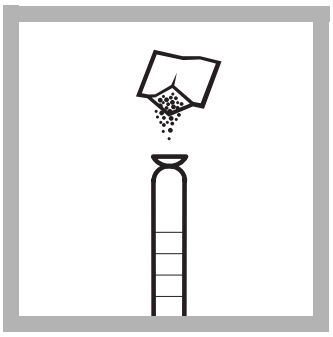
Select program

351 N, Nitrate LR.

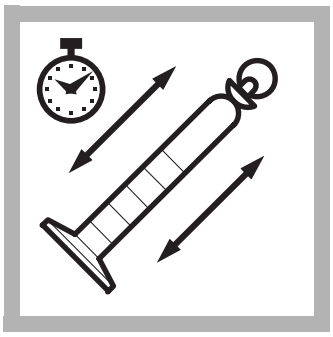
Touch **Start**.



2. Fill a 25-mL graduated mixing cylinder with 15 mL of sample.



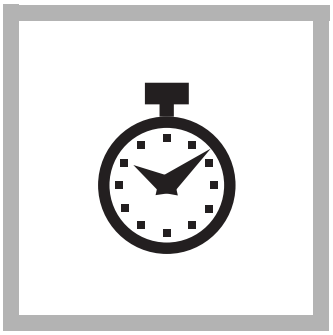
3. Add the contents of one NitraVer 6 Reagent Powder Pillow to the cylinder. Stopper.



4. Touch the timer icon.

Touch **OK**.

Shake the cylinder vigorously for three minutes.



5. When the timer beeps, touch the timer icon again.

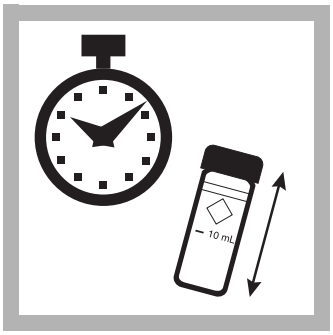
Touch **OK**.

A 2-minute reaction period will begin.

6. When the timer beeps, carefully pour 10 mL of the sample into a clean, round sample cell. Do not transfer any cadmium particles to the sample cell.

7. Add the contents of one NitriVer3 Nitrite Reagent Powder Pillow to the sample cell (this is the prepared sample).

Cap the sample cell.

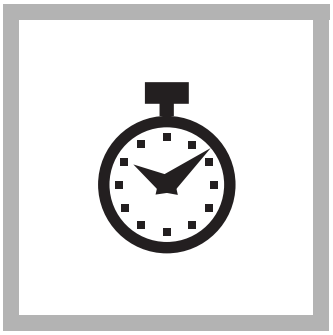


8. Touch the timer icon.

Touch **OK**.

Shake the sample cell gently for 30 seconds.

A pink color will develop if nitrate is present.



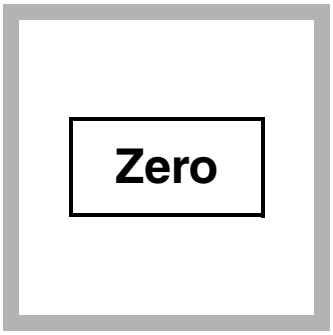
9. Touch the timer icon.

A 15-minute reaction period will begin.

10. When the timer beeps, fill a second sample cell with 10 mL of original sample (this is the blank).

Cap the sample cell.

11. Place the blank into the cell holder.

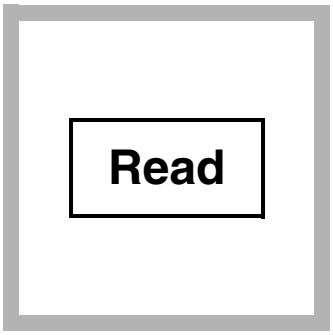


12. Touch **Zero**.

The display will show:

0.00 mg/L NO_3^-–N

13. Place the prepared sample into the cell holder.



14. Touch **Read**.

Results will appear in mg/L NO_3^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	100 mg/L
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration.
Ferric iron	All levels
Nitrite	All levels: This method measures both the nitrate and nitrite in the sample. If nitrite is present, the nitrite nitrogen test (Program #371) should be done on the sample. Pretreat the nitrate nitrogen sample with the following pretreatment. Then subtract the amount of nitrite found from the results of the LR nitrate nitrogen test. **1.** Add 30-g/L Bromine Water (Cat. No. 2211-20) dropwise to the sample in *step 3* until a yellow color remains. Mix after each drop. **2.** Add one drop of 30-g/L Phenol Solution (Cat. No. 2112-20) to destroy the color. **3.** Proceed with the LR Nitrate procedure.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage and Preservation

More reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 48 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (Cat. No. 979-49) per liter and store at 4 °C.

Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-53). Do not use mercury compounds as preservatives. Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Nitrate Nitrogen Voluette® Ampule Standard, 12.0-mg/L NO_3^-–N (Cat. No. 14333-10).
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 15 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. To test accuracy, use a 0.20-mg/L NO_3^-–N standard in place of the sample and perform the procedure as described. Prepare this standard by diluting 2.00 mL of a 10-mg/L Nitrate Nitrogen Standard Solution to 100.0 mL with deionized water.
2. To adjust the calibration curve using the reading obtained with the 0.20-mg/L nitrate nitrogen standard, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.2 mg/L NO_3^-–N

Program	95% Confidence Limits of Distribution
351	0.18–0.23 mg/L NO_3^-–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.004 mg/L NO_3^-–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with chromotropic acid to form a pink-colored product. Test results are measured at 507 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Low Range Nitrate Reagent Set (100 tests)			24298-00
Includes:			
NitraVer® 6 Nitrate Reagent Powder Pillows	1 pillow	100/pkg	21072-49
NitriVer® 3 Nitrite Reagent Powder Pillows	1 pillow	100/pkg	21071-69

Required Apparatus

Cylinder, graduated, mixing, 25-mL	1	each	20886-40
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Nitrate Nitrogen Standard Solution, 10.0-mg/L NO_3^-–N		500 mL	307-49
Nitrate Nitrogen Standard Solution, Voluette® Ampule, 12-mg/L NO_3^-–N		16/pkg	14333-10
Water, deionized		4 liters	272-56

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WORLD HEADQUARTERS
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3/04 1ed

Method 10020 **Chromotropic Acid Method**

Test 'N Tube™ Vials **HR (0.2 to 30.0 mg/L NO_3^-–N)**

Scope and Application: For water and wastewater

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water (nitrate-free) in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Test 'N Tube Method 10020

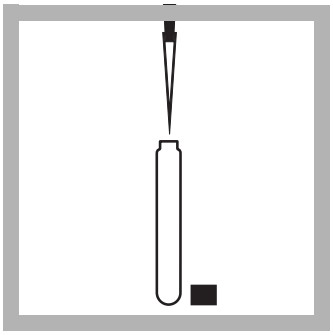
1. Touch

Hach Programs.

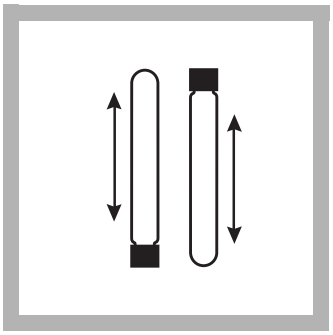
Select program

344 N, Nitrate HR TNT.

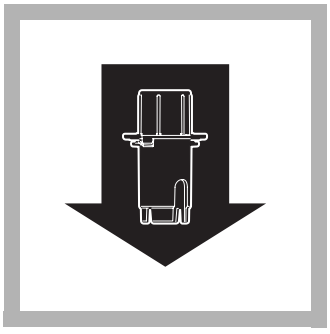
Touch **Start**.



2. Remove the cap from a NitraVer X Reagent A Test 'N Tube vial and add 1.00 mL of sample (this is the blank).



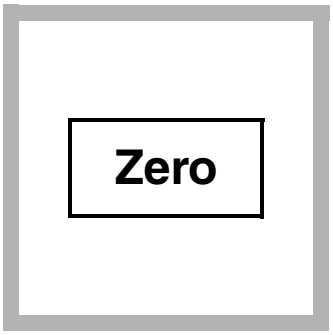
3. Cap the tube and invert ten times to mix.



4. Install the 16-mm adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for installation details.*

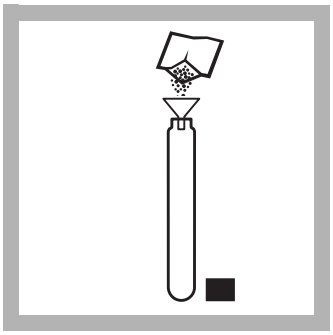
Insert the blank into the adapter.



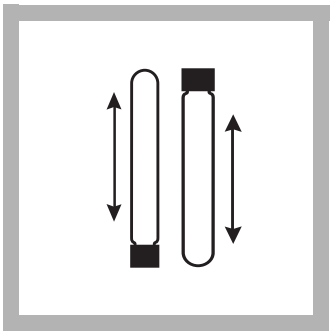
5. Touch **Zero**.

The display will show:

0.0 mg/L NO_3^-–N

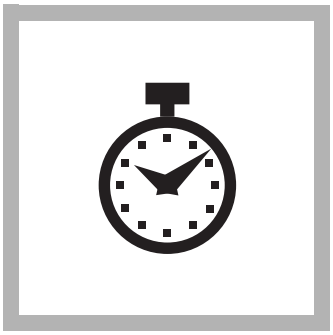


6. Remove the vial from the instrument. Using a funnel, add the contents of one NitraVer X Reagent B Powder Pillow to the vial.



7. Cap and invert ten times to mix (this is the *prepared sample*).

Some solid matter will not dissolve.

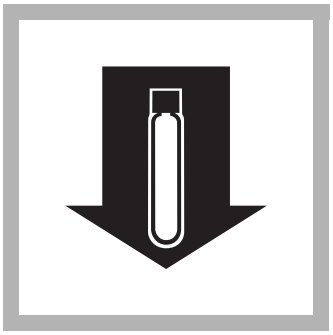


8. Touch the timer icon.

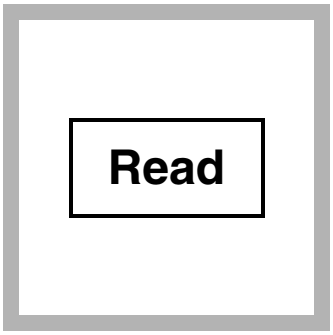
Touch **OK**.

A five-minute reaction period will begin. Do not invert the vial again.

A yellow color will develop if nitrate is present.



9. Within five minutes after the timer beeps, wipe the prepared sample and place it into the cell holder.



10. Touch **Read**.

Results will appear in mg/L NO_3^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Barium	A negative interference at concentrations greater than 1 mg/L.
Chloride	Does not interfere below 1000 mg/L.
Nitrite	A positive interference at concentrations greater than 12 mg/L. Remove nitrite interference up to 100 mg/L by adding 400 mg (one full 0.5 g Hach measuring spoon) of Urea (Cat. No. 11237-26) to 10 mL of sample. Swirl to dissolve. Proceed with the nitrate test as usual.
Copper	Positive at all levels.

Sample Collection, Preservation, and Storage

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods (up to 14 days), adjust sample pH to 2 or less with Concentrated Sulfuric Acid, ACS (about 2 mL per liter) (Cat. No. 979-49). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-26).

Do not use mercury compounds as preservatives.

Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a High Range Nitrate Nitrogen Voluette® Ampule Standard, 500 mg/L NO_3^-–N.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Use a 10.0-mg/L Nitrate Nitrogen Standard Solution to check test accuracy.

See *Section 3.2.1 Standard Solutions* on page *31* for more information.

Method Performance

Precision

Standard: 10.0 mg/L NO_3^-–N

Program	95% Confidence Limits of Distribution
344	9.5–10.5 mg/L NO_3^-–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.2 mg/L NO_3^-–N

See for more information.

Summary of Method

Nitrate in the sample reacts with chromotropic acid under strongly acidic conditions to yield a yellow product with a maximum absorbance at 410 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Test 'N Tube NitraVer® X Nitrate Reagent Set (50 tests)			26053-45

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Funnel, micro, poly	1	each	25843-35
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	varies	50/pkg	21856-96
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Test Tube Rack, cooling	1–3	each	18641-00

Required Standards

Nitrate Nitrogen Standard Solution, 10-mg/L N		500 mL	307-49
Nitrate Nitrogen Standard Solution, Voluette® Ampule, 500-mg/L N		16/pkg	14260-10
Water, deionized		4 liters	272-56

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Dimethylphenol

UniCell™ Vials
Scope and Application: For water and wastewater

(1.0 to 60.0 mg/L NO_3)
(0.2 to 13.5 mg/L NO_3^-–N)

Tips and Techniques

- Adjust the pH of preserved samples to between pH 3–10 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results (see *Accuracy Check)*.
- See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for information on adjusting the calibration curve.
- Make sure that the temperature of the sample and the reagents is between 20 and 24 °C. Higher temperatures can cause high-bias results; lower temperatures can cause low-bias results.

UniCell Vials

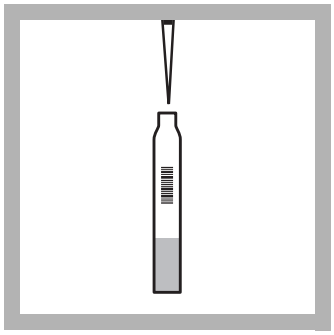
1. Touch

Hach Programs.

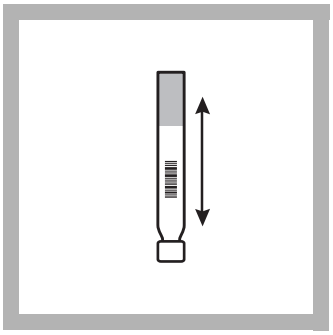
Select program

805 Nitrate, HCT 106.

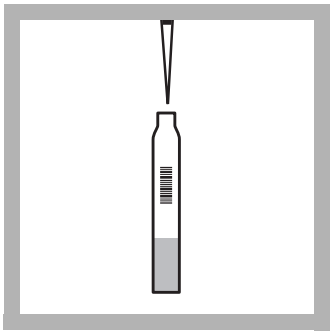
Touch **Start**.



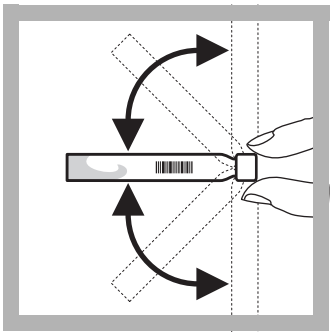
2. Pipet 0.2 mL of Dimethylphenol Solution A (HCT 106 A) into a sample vial.



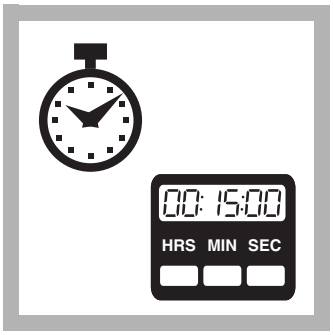
3. Cap the vial and invert 3 times to mix.



4. Immediately pipet 1.0 mL of sample into the vial.



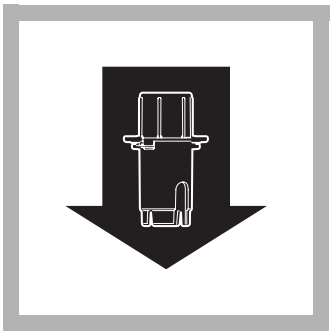
5. Cap the sample vial and invert a few times until the reagents are mixed and appear uniform.



6. Touch the timer icon.

Touch **OK**.

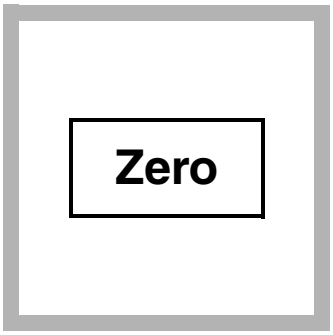
A 15-minute reaction period will begin.



7. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Wipe the zeroing vial (**white** cap) and place it into the cell adapter.

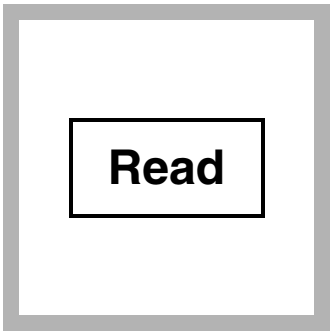


8. Touch **Zero**.

The display will show:

0.0 mg/L NO_3

9. After the timer beeps, wipe the sample vial and place it into the cell holder.



10. Touch **Read**.

Results will appear in mg/L NO_3.

Interferences

The ions and substances listed in the following tables have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions and substances have not been evaluated.

Ion	No interference up to:
K^+, Na^+, Cl^-	500 mg/L
COD	200 mg/L
Ag^+	100 mg/L

Ion	No interference up to:
Pb^{2+}, Zn^{2+}, Ni^{2+}, Hg^{2+}, Fe^{3+}, Cd^{2+}, Sn^{2+}, Ca^{2+}, Cu^{2+}	50 mg/L
Co^{2+}, Fe^{2+}	10 mg/L
Cr^{6+}	5 mg/L
NO_2^-	2 mg/L

Interfering Substance	Interferes above:
COD	200 mg/L (high bias)
Nitrite	2 mg/L (high bias)
Chloride	500 mg/L (low bias)

Sample Collection, Preservation, and Storage

Analyze samples within three hours after collection for best results. Collect samples in clean plastic or glass bottles. Store in a cool place. Warm to room temperature before performing the test. For longer storage periods, add 1 mL of concentrated sulfuric acid per liter and store at 4 °C.

Before testing the stored sample, warm to room temperature and adjust the pH to 3–10 with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-26).

Do not use mercury compounds as preservatives. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Enter 4427 mg/L NO_3 (1000-mg/L NO_3–N) as the standard concentration.
4. Touch **OK** to accept the default values for sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 20886-42) with 100 mL of sample. Use a pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 6-mg/L NO_3–N standard solution by pipetting 6 mL of 1000-mg/L NO_3–N into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the nitrate procedure as described above.

Note: *Make sure the selected chemical form is NO_3–N.*

Method Performance

Precision

Standard: 5.7 mg/L NO_3–N

Program	95% Confidence Limits of Distribution
805	5.3–6.1 mg/L NO_3–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
805	Entire range	0.010	0.1 mg/L NO_3–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Nitrate ions react with 2,6-dimethylphenol in a solution containing sulfuric and phosphoric acids to form 4-nitro-2,6-dimethylphenol. Measurements are taken at 370 nm.

Required Reagents

Description	Unit	Cat. No.
Nitrate - NO_3, UniCell™ HCT 106	23/pkg	HCT 106

Optional Reagents

EDTA Solution	50 mL SCDB	22419-26
Nitrate Nitrogen Standard Solution, 1000-mg/L as NO_3–N	500 mL	12792-49
Sodium Hydroxide Standard Solution	50 mL SCDB	2450-26
Sulfuric Acid, ACS	500 mL	979-49

Optional Apparatus

Cylinder, mixing, 100-mL	each	508-42
Flask, volumetric, 1000-mL	each	14574-53
Pipettor, Jencons, 100–1000 µL	each	27949-00
Replacement Tips, for 27949-00	400/pkg	27950-00
Pipettor, Jencons, 1–5 mL	each	27951-00
Replacement Tips, for 27951-00	100/pkg	27952-00

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★Method 8507 **Diazotization Method**

Powder Pillows or AccuVac® Ampuls **LR (0.002 to 0.300 mg/L NO_2^-–N)**

Scope and Application: For water, wastewater, and seawater; USEPA approved for wastewater analysis*

* Federal Register, 44(85), 25505 (May 1, 1979)

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows Method 8507

1. Touch

Hach Programs.

Select program

371 N, Nitrite LR.

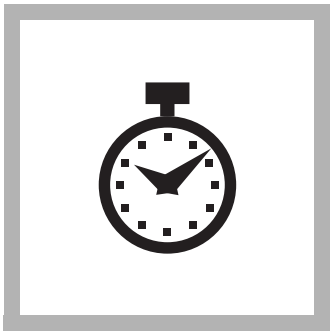
Touch **Start**.

2. Fill a round sample cell with 10 mL of sample.

3. Add the contents of one NitriVer 3 Nitrite Reagent Powder Pillow (this is the prepared sample).

Cap and shake to dissolve.

A pink color will develop if nitrite is present.



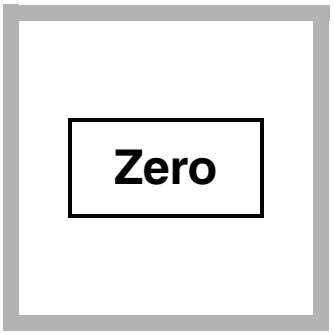
4. Touch the timer icon.

Touch **OK**.

A 20-minute reaction period will begin.

5. When the timer beeps, fill a second sample cell with 10 mL of sample (this is the blank).

6. Wipe the blank and place it into the cell holder.



7. Touch **Zero**.

The display will show:

0.000 mg/L NO_2^-–N

8. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L NO_2^-–N.

AccuVac® Ampul — Method 8507

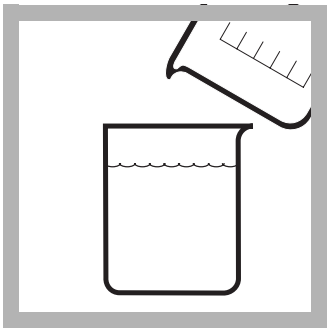
1. Touch

Hach Programs.

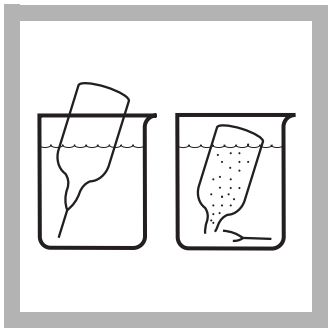
Select program

375 N, Nitrite LR AV.

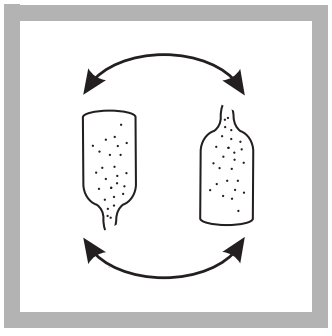
Touch **Start**.



2. Pour at least 40 mL of sample into a 50-mL beaker.

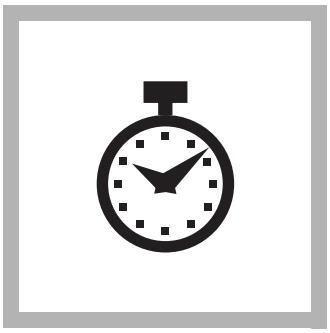


3. Fill a NitriVer 3 Nitrite AccuVac® Ampul with sample. Keep the tip immersed while the ampule fills.



4. Invert the ampule several times to mix.

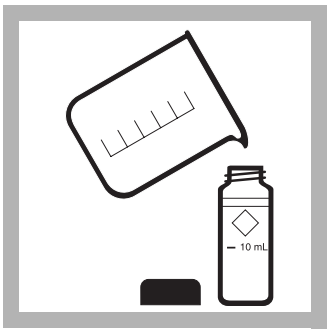
A pink color will develop if nitrite is present.



5. Touch the timer icon.

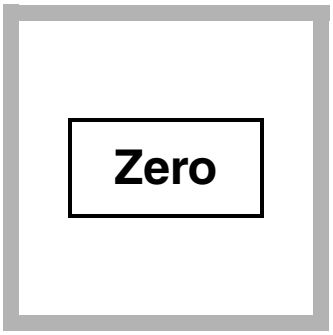
Touch **OK**.

A 20-minute reaction period will begin.



6. When the timer beeps, fill a sample cell with at least 10 mL of sample (this is the blank).

7. Wipe the blank and place it into the cell holder.

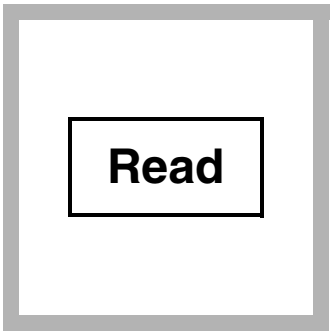


8. Touch **Zero**.

The display will show:

0.000 mg/L NO_2^-–N

9. Wipe the AccuVac Ampul and place it into the cell holder. Read the results.



10. Touch **Read**.

Results will appear in mg/L NO_2^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Antiminous ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Store at 4 °C (30 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. Do not use acid preservatives.

Accuracy Check

Standard Solution Method

Preparing nitrite standards is difficult. A standard should be prepared by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater,* Method 4500—NO_2-B (p. 4–86 of 18th edition). Prepare a 0.150-mg/L standard.

Method Performance

Precision
Standard: 0.150 mg/L NO_2^-–N

Program	95% Confidence Limits of Distribution
371	0.146–0.154 mg/L NO_2^-–N
375	0.140–0.160 mg/L NO_2^-–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
371	Entire range	0.010	0.002 mg/L NO_2^-–N
375	Entire range	0.010	0.002 mg/L NO_2^-–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. Test results are measured at 507 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
NitriVer® 3 Nitrite Reagent Powder Pillows	1 pillow	100/pkg	21071-69
or			
NitriVer® 3 Nitrite Reagent AccuVac® Ampul	1 ampul	25/pkg	25120-25

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Sodium Nitrite, ACS	454 g	2452-01
Water, deionized	4 liters	272-56

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On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

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Method 8153 **Ferrous Sulfate Method***

Powder Pillows **HR (2 to 250 mg/L NO_2^-)**

Scope and Application: For water and wastewater

* Adapted from McAlpine, R. and Soule, B., *Qualitative Chemical Analysis*, New York, 476, 575 (1933)

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- After adding the reagent, a greenish-brown color will develop if nitrite is present.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows Method 8153

1. Touch

Hach Programs

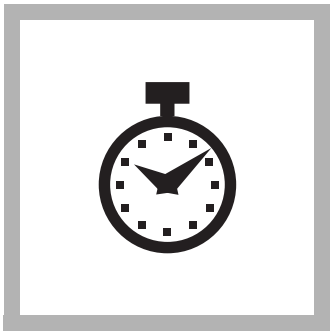
Select program

373 N, Nitrite HR.

Touch **Start**.

2. Fill a round sample cell with 10 mL of sample.

3. Add the contents of one NitriVer® 2 Nitrite Reagent Powder Pillow. Cap and shake to dissolve (this is the prepared sample).



4. Touch the timer icon.

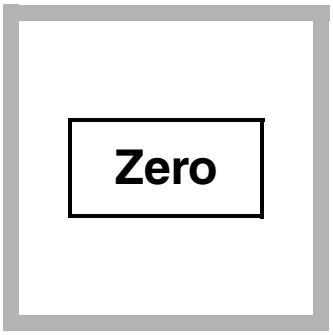
Touch **OK**.

A ten-minute reaction period will begin.

To prevent low results, leave the sample on a flat surface and **do not disturb it during the reaction period.**

5. Fill another sample cell with 10 mL of sample (this is the blank).

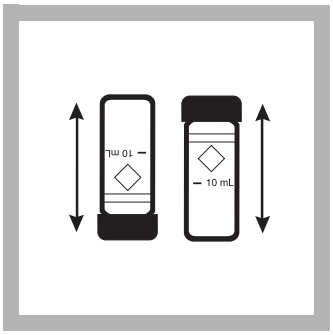
6. Wipe the blank and place it into the cell holder.



7. Touch **Zero**.

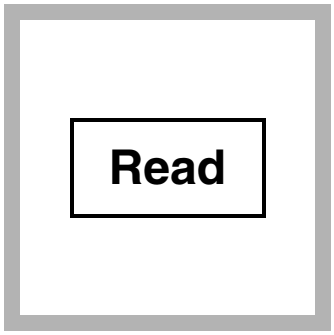
The display will show:

0 mg/L NO_2^-



8. After the timer beeps, gently invert the prepared sample twice. Avoid excessive mixing, or low results may occur.

9. Wipe the prepared sample and place it into the cell holder.



10. Touch **Read**.

Results will appear in mg/L NO_2^-.

Interferences

This test does not measure nitrates nor is it applicable to glycol-based samples. Dilute glycol-based samples and follow the Low Range Nitrite procedure.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles.

The following storage instructions are necessary only when prompt analysis is impossible. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. Do not use acid preservatives.

Accuracy Check

Standard Solution Method

Preparing nitrite standards is difficult. A standard should be prepared by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*. Prepare a 200-mg/L standard using Sodium Nitrite, ACS (Cat. No. 2452-01).

Method Performance

Precision

Standard: 200 mg/L NO_2^-

Program	95% Confidence Limits of Distribution
373	193–207 mg/L NO_2^-

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	1.6 mg/L NO_2^-

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The method uses ferrous sulfate in an acidic medium to reduce nitrite to nitrous oxide. Ferrous ions combine with the nitrous oxide to form a greenish-brown complex in direct proportion to the nitrite present. Test results are measured at 585 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
NitriVer® 2 Nitrite Reagent Powder Pillows	1 pillow	100/pkg	21075-69
Water, Deionized	4 liters		272-56

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

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Nitrite

Method 10019 **Diazotization Method**

Test 'N Tube™ Vials **LR (0.003 to 0.500 mg/L NO_2^-–N)**

Scope and Application: For water, wastewater, and seawater

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Test 'N Tube Method 10019

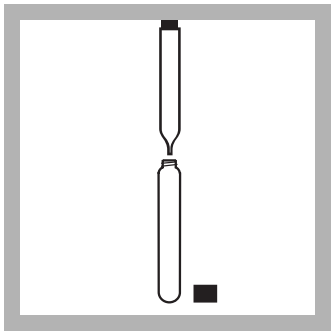
1. Touch

Hach Programs.

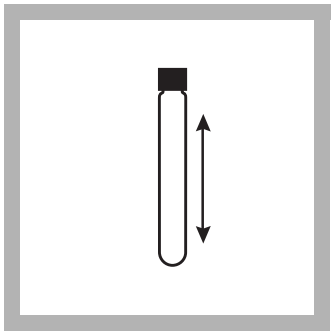
Select program

345 N, Nitrite LR TNT.

Touch **Start**.

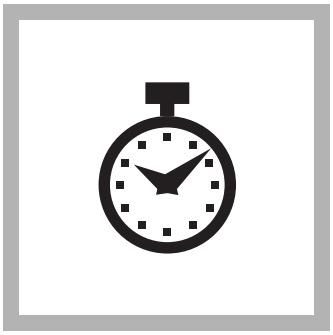


2. Fill a Test 'N Tube NitriVer® 3 Nitrite vial with 5 mL of sample.



3. Cap and shake to dissolve the powder (this is the prepared sample).

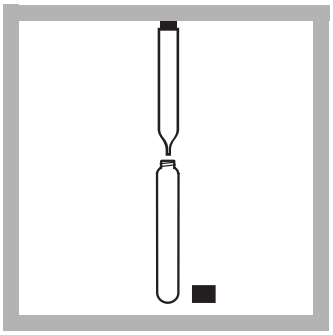
A pink color will develop if nitrite-nitrogen is present.



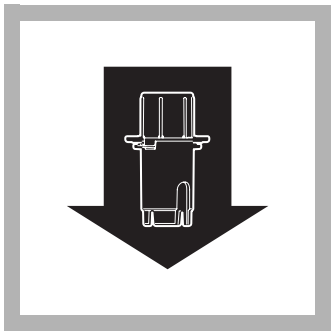
4. Touch the timer icon.

Touch **OK**.

A 20-minute reaction period will begin.



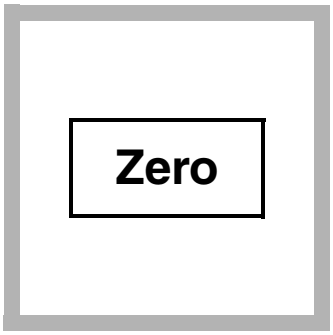
5. When the timer beeps, fill an empty Test 'N Tube™ vial with 5 mL of sample (this is the blank).



6. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

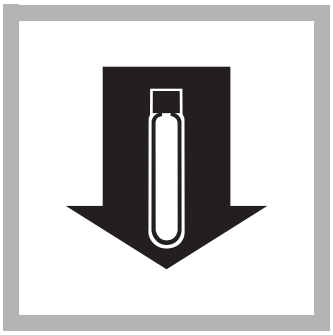
Wipe the blank and place it into the adapter.



7. Touch **Zero**.

The display will show:

0.000 mg/L NO_2^-–N



8. Place the prepared sample cell into the cell holder.

Touch **Read**.

Results will appear in mg/L NO_2^-–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Antiminous ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interfere at all levels

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Store at 4 °C (30 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test.

Accuracy Check

Standard Solution Method

Preparing nitrite standards is difficult. A standard should be prepared by a trained chemist. Hach recommends using the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, 18th ed., under the headings "Stock nitrite solution," "Intermediate nitrite solution," and "Standard nitrite solution." Prepare a 0.150-mg/L standard. Perform the nitrite test on the standard solution.

Method Performance

Precision

Standard: 0.150 mg/L NO_2^-–N

Program	95% Confidence Limits of Distribution
345	0.146–0.154 mg/L NO_2^-–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.003 mg/L NO_2^-–N

See Section 3.4.5 Sensitivity on page *44* for more information.

Summary of Method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. Test results are measured at 507 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
NitriVer® 3 Low Range Nitrite Test 'N Tube™ Vial Set (50 tests)			26083-45

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	varies	50/pkg	21997-96

Required Standards

Sodium Nitrite, ACS		454 g	2452-01
Water, deionized		4 liters	272-56

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Nitrite

Diazotization Method*

UniCell™ Vials

(0.020 to 0.600 mg/L NO_2^-–N)

Scope and Application: For water, wastewater, and seawater

* Reagent sets for this method are only available in Europe.

Tips and Techniques

- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method for a proof of accuracy. (See *Accuracy Check*).
- See *3.2.4* on page *40* for information on calibration curve adjustment.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

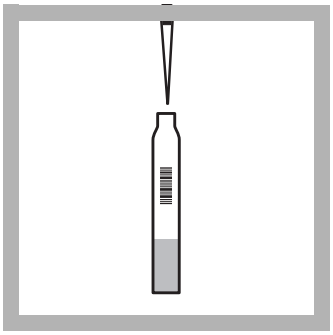
1. Touch

Hach Programs.

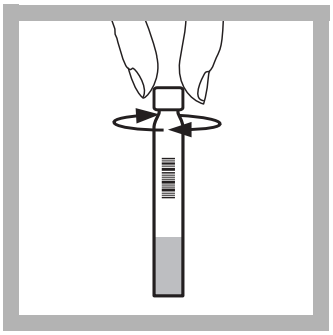
Select program

807 Nitrite, HCT 116

Touch **Start**.

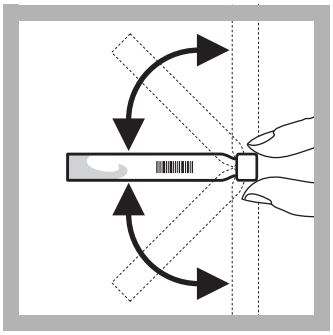


2. Pipet 4.0 mL of sample into a sample vial.

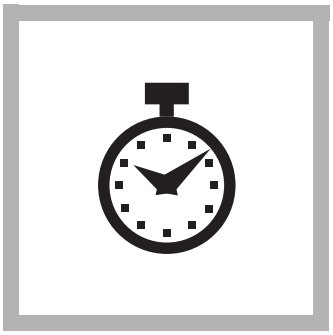


3. Immediately screw a **green** UniCap A (HCT 116 A) onto the sample vial.

Close the UniCap bottle immediately after use.

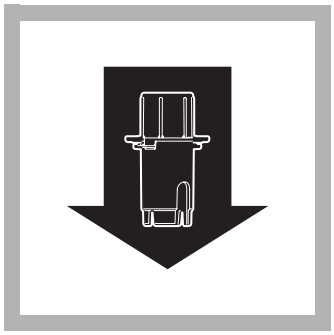


4. Invert the sample vial repeatedly until the reagent in the cap is completely dissolved.



5. Touch the timer icon.

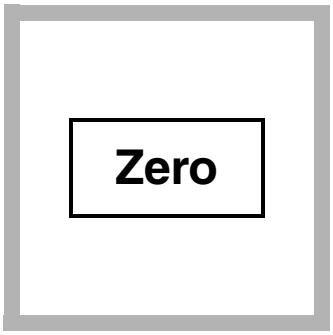
A 10-minute reaction period will begin. Invert the sample vial again after the reaction period has elapsed.



6. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

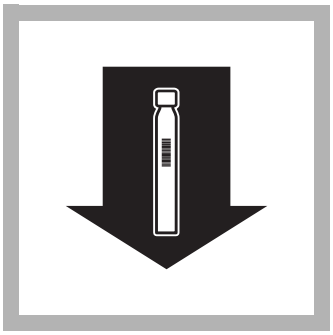
Wipe the zero vial (**white** cap) and place it into the cell.



7. Touch **Zero**.

The display will show:

0.000 mg/L NO_2^-–N
Underrange



8. Wipe the sample vial and place it into the celladapter.

Touch **Read**.

Results will appear in mg/L NO_2^-–N.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	Interference Levels
Cl^-, SO_4^{2-}	2000 mg/L
K^+, NO_3^-, Ca^{2+}	1000 mg/L
NH_4^+, PO_4^{3-}	500 mg/L
Mg^{2+}	100 mg/L
Cr^{3+}, Hg^{2+}	50 mg/L
Co^{2+}, Zn^{2+}, Cd^{2+}	25 mg/L
Fe^{3+}, Ni^{2+}	12 mg/L
Ag^+, Fe^{2+}	10 mg/L
Sn^{2+}	5 mg/L

Chromium (VI) ions interfere. Copper (II) ions interfere even at concentrations below 1 mg/L.

Sample Collection, Storage, and Preservation

If prompt analysis is impossible, store sample for 24 to 48 hours at 4 °C (39 °F) or lower. Warm sample to room temperature before testing. Do not use acid preservation.

Accuracy Check

Standard Solution Method

Prepare a 0.25-mg/L NO_2^-–N standard solution by pipetting 1.00 mL of 250-mg/L NO_2^-–N standard into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the nitrite procedure as described.

Method Performance

Precision

Standard: 0.300 mg/L NO_2^-–N

Program	95% Confidence Limits of Distribution
807	0.230–0.370 mg/L NO_2^-–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
807	Entire range	0.010	0.1 mg/L NO_2^-–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Nitrites react with primary aromatic amines in an acidic solution to form diazonium salts. These salts combine with aromatic compounds that contain an amino group or a hydroxyl group to form intensely colored azo dyes. Test results are measured at 515 nm.

Required Reagents

Description	Unit	Cat. No.
Nitrite - (NO_2^-–N), UniCell™ HCT 116*	23/pkg	HCT 116

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Flask, volumetric, 50-mL	each	14574-41
Flask, volumetric, 100-mL	each	14574-42
Flask, volumetric, 1000-mL	each	14574-53
Graduated cylinder, mixing, 100-mL	each	20886-42

Optional Standards

Nitrite Standard Solution, 250-mg/L	500 mL	23402-49

* Available in Europe only

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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

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★Method 8038

Nessler Method*

(0.02 to 2.50 mg/L NH_3–N)

Scope and Application: For water, wastewater, and seawater; distillation is required for wastewater and seawater; USEPA accepted for wastewater analysis (distillation required); see *Distillation* following this procedure.

* Adapted from *Standard Methods for the Examination of Water and Wastewater 4500-NH_3 B & C*.

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Nessler Reagent is toxic and corrosive. Pipet carefully, using a pipet filler.
- When dispensing reagent from a dropper bottle, hold the bottle vertically. Do not hold the bottle at an angle.
- Nessler Reagent contains mercuric iodide. Both the sample and the blank will contain mercury (D009) at a concentration regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. See *Section 4* for further information on proper disposal of these materials.
- If using the Flow Cell, periodically clean the cells by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel. Flush with enough deionized water to dissolve. Rinse the cell thoroughly.
- A yellow color will develop if ammonia is present. (The reagent will cause a faint yellow color in the blank.)

Method 8038

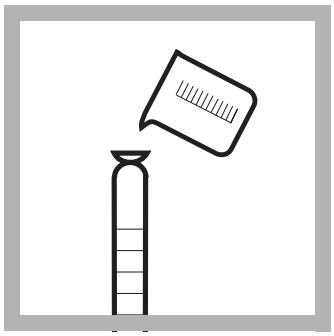
1. Touch

Hach Programs.

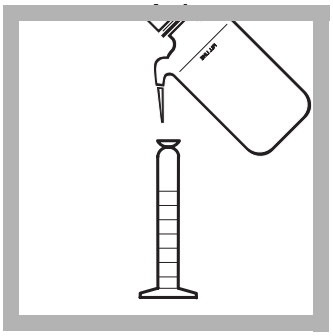
Select program

380 N, Ammonia, Ness.

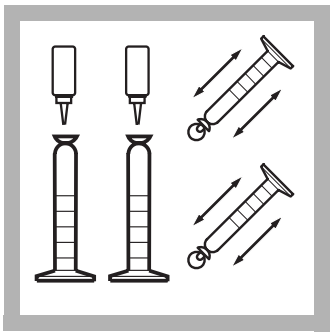
Touch **Start**.



2. Fill a 25-mL mixing graduated cylinder to the 25-mL mark with sample (this is the prepared sample).

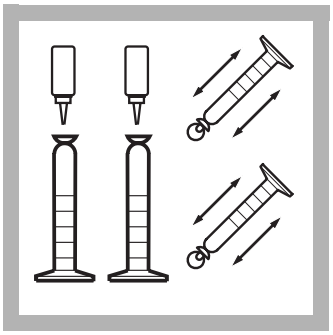


3. Fill a 25-mL mixing graduated cylinder to the 25-mL mark with deionized water (this is the blank).



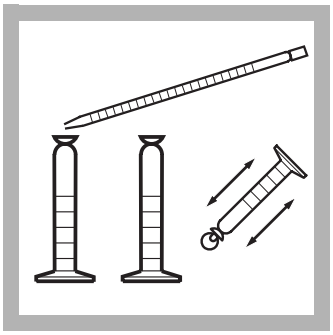
4. Add three drops of Mineral Stabilizer to each cylinder.

Stopper and invert several times to mix.



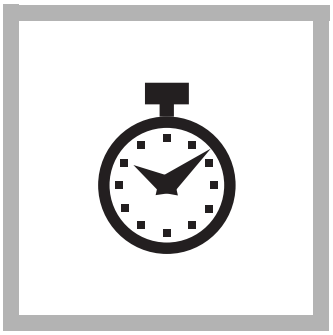
5. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder.

Stopper and invert several times to mix.



6. Pipet 1.0 mL of Nessler Reagent into each cylinder.

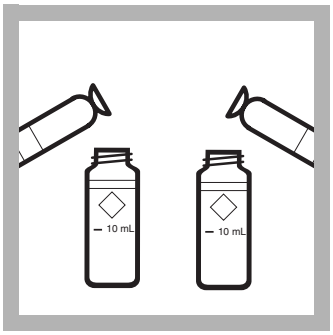
Stopper and invert several times to mix.



7. Touch the timer icon.

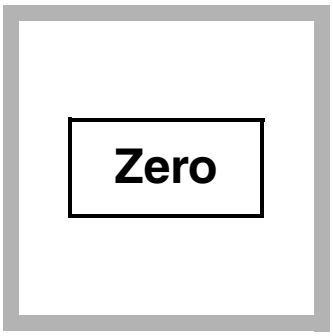
Touch **OK**.

A one-minute reaction period will begin.



8. Pour each solution into a round sample cell.

9. When the timer beeps, place the blank into the cell holder.

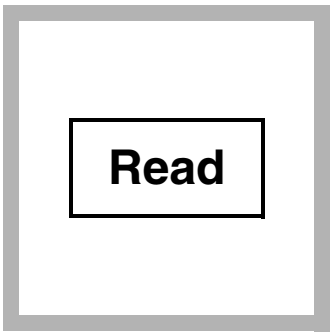


10. Touch **Zero**.

The display will show:

0.00 mg/L NH_3 –N

11. Wipe the prepared sample and place it into the cell holder.



12. Touch **Read.**

Results will appear in mg/L NH_3–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Chlorine	Remove residual chlorine by adding 2 drops of sodium arsenite for each mg/L Cl from a 250 mL sample. Sodium thiosulfate can be used in place of sodium arsenite. See *Sample Collection, Storage, and Preservation* below.
Hardness	A solution containing a mixture of 500 mg/L $CaCO_3$ and 500 mg/L Mg as $CaCO_3$ does not interfere. If the hardness concentration exceeds these concentrations, add extra Mineral Stabilizer.
Iron	Interferes at all levels by causing turbidity with Nessler Reagent.
Seawater	May be analyzed by adding of 1.0 mL (27 drops) of Mineral Stabilizer to the sample before analysis. This complexes the high magnesium concentrations found in sea water, but the sensitivity of the test is reduced by 30 percent due to the high chloride concentration. For best results, perform a calibration, using standards spiked to the equivalent chloride concentration, or distill the sample as described below.
Sulfide	Interferes at all levels by causing turbidity with Nessler Reagent.
Glycine, various aliphatic and aromatic amines, organic chloramines, acetone, aldehydes and alcohols	May cause greenish or other off colors or turbidity. Distill the sample if these compounds are present.

Sample Collection, Storage, and Preservation

Collect samples in clean glass or plastic bottles. If chlorine is present, add one drop of 0.1 N Sodium Thiosulfate (Cat. No. 323-32) for each 0.3 mg/L Cl_2 in a 1-liter sample. Preserve the sample by reducing the pH to 2 or less with sulfuric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature and neutralize with 5 N Sodium Hydroxide (Cat. No. 2450-32) before analysis. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Nitrogen Ammonia Voluette® Ampule Standard, 50-mg/L NH_3–N.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of the 50 mg/L standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, the display will show the extrapolated concentration value and the "best-fit" line through the standard additions data points, accounting for matrix interferences.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

1. To check accuracy, use a 1.0-mg/L Nitrogen Ammonia Standard Solution. Or, prepare a 1.0-mg/L ammonia nitrogen standard solution by pipetting 1.00 mL of Nitrogen Ammonia Voluette® Ampule Standard, 50-mg/L, into a 50-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the Nessler procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L Nitrogen Ammonia Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 1.00 mg/L NH_3–N

Program	95% Confidence Limits of Distribution
380	0.93–1.07 mg/L NH_3–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.02 mg/L NH_3–N2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Distillation

1. Measure 250 mL of sample into a 250-mL graduated cylinder and pour into a 400-mL beaker. Destroy chlorine, if necessary, by adding 2 drops of Sodium Arsenite Solution per mg/L Cl_2.
2. Add 25 mL of Borate Buffer Solution and mix. Adjust the pH to about 9.5 with 1 N sodium hydroxide solution. Use a pH meter.
3. Set up the General Purpose Distillation Apparatus (Cat. No. 22653-00) as shown in the *Hach Distillation Apparatus Manual*. Pour the solution into the distillation flask. Add a stir bar.
4. Use a graduated cylinder to measure 25 mL of deionized water into a 250-mL Erlenmeyer flask. Add the contents of one Boric Acid Powder Pillow. Mix thoroughly. Place the flask under the still drip tube. Elevate so the end of the tube is immersed in the solution.

5. Turn on the heater power switch. Set the stir control to 5 and the heat control to 10. Turn on the water and adjust to maintain a constant flow through the condenser.
6. Turn off the heater after collecting 150 mL of distillate. Immediately remove the collection flask to avoid sucking solution into the still. Measure the distillate to ensure 150 mL was collected (total volume = 175 mL).
7. Adjust the pH of the distillate to about 7 with 1 N sodium hydroxide. Use a pH meter.
8. Pour the distillate into a 250-mL volumetric flask; rinse the Erlenmeyer with deionized water. Add the rinsings to the volumetric flask. Dilute to the mark. Stopper. Mix thoroughly. Analyze as described above.

Summary of Method

The Mineral Stabilizer complexes hardness in the sample. The Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonium ions. A yellow color is formed proportional to the ammonia concentration. Test results are measured at 425 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Ammonia Nitrogen Reagent Set			24582-00
Includes:			
Nessler Reagent	2 mL	500 mL	21194-49
Mineral Stabilizer	6 drops	50 mL SCDB	23766-26
Polyvinyl Alcohol Dispersing Agent	6 drops	50 mL SCDB	23765-26
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Cylinder, graduated, mixing, 25-mL	2	each	21190-40
Pipet, serological, 1-mL	2	each	9190-02
Pipet Filler, safety bulb	1	each	14651-00
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Nitrogen, Ammonia Standard Solution, 1-mg/L NH_3–N	500 mL	1891-49
Nitrogen, Ammonia Standard Sol., 10-mL Voluette® Ampule, 50-mg/L NH_3–N	16/pkg	14791-10

Distillation Reagents

Borate Buffer Solution	1000 mL	14709-53
Boric Acid Powder Pillows	100/pkg	14817-99
Sodium Arsenite Solution, 5.0-g/L	100 mL MDB	1047-32
Sodium Hydroxide Standard Solution, 1.0 N	100 mL MDB	1045-32

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Nitrogen, Ammonia

Method 8155 **Salicylate Method***

Powder Pillows **(0.01 to 0.50 mg/L NH_3–N)**

Scope and Application: For water, wastewater, and seawater

* Adapted from *Clin. Chim. Acta., 14*, 403 (1966)

Tips and Techniques

• A green color will develop if ammonia nitrogen is present.

Powder Pillows Method 8155

1. Touch

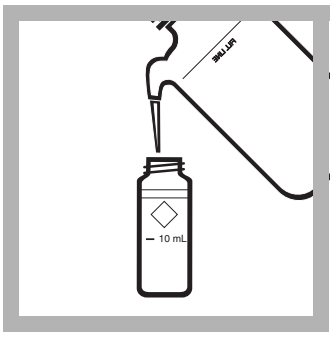
Hach Programs.

Select program

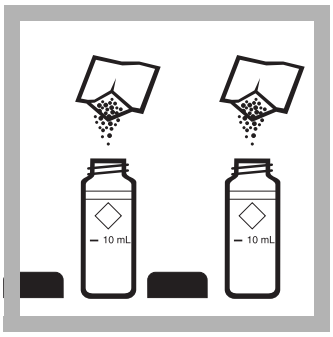
385 N, Ammonia, Salic.

Touch **Start**.

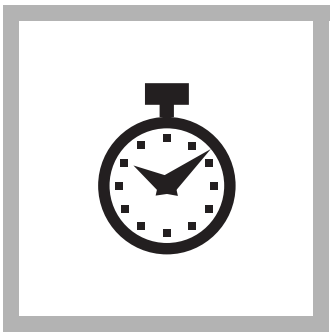
2. Fill a round sample cell to the 10-mL mark with sample.



3. Fill another round sample cell to the 10-mL mark with deionized water (this is the blank).



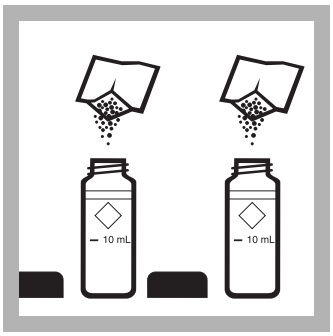
4. Add the contents of one Ammonia Salicylate Powder Pillow to each cell. Stopper and shake to dissolve the powder.



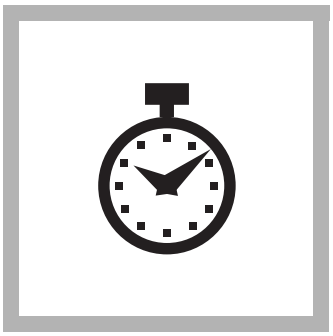
5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.



6. When the timer beeps, add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each cell. Stopper and shake to dissolve the reagent.

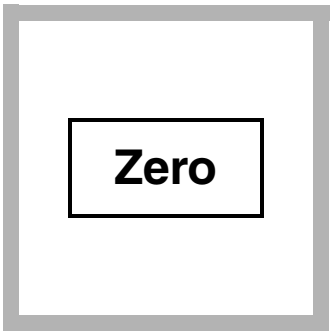


7. Touch the timer icon. Touch **OK**.

A 15-minute reaction period will begin.

A green color will develop if ammonia-nitrogen is present.

8. When the timer beeps, place the blank into the cell holder.

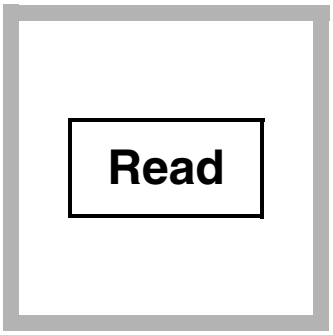


9. Touch **Zero**.

The display will show:

0.00 mg/L NH_3–N

10. Wipe the sample and place it into the cell holder.



11. Touch **Read.**

Results will appear in mg/L NH_3–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	Greater than 1000 mg/L as $CaCO_3$
Iron	All levels. Correct for iron interference as follows: **1.** Determine the amount of iron present in the sample by following one of the Iron, Total, procedures. **2.** Add the same iron concentration to the ammonia-free water in step 3. The interference will be successfully blanked out.
Magnesium	Greater than 6000 mg/L as $CaCO_3$
Nitrate	Greater than 100 mg/L as NO_3^-–N
Nitrite	Greater than 12 mg/L as NO_2^-–N
Phosphate	Greater than 100 mg/L as PO_4^{3-}–P
Sulfate	Greater than 300 mg/L as SO_4^{2-}
Sulfide	Sulfide will intensify the color. Eliminate sulfide interference as follows: **1.** Measure about 350 mL of sample in a 500-mL Erlenmeyer flask (Cat. No. 505-49). **2.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow (Cat. No. 2418-99). Swirl to mix. **3.** Filter the sample through a Folded Filter Paper (Cat. No. 1894-57) and Filter Funnel (Cat. No. 1083-67). **4.** Use the filtered solution in step 3.
Other Substances	Less common interferences such as **hydrazine** and **glycine** will cause intensified colors in the prepared sample. **Turbidity** and **color** will give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set (Cat. No. 22653-00).

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.

If chlorine is known to be present, the sample must be treated immediately with sodium thiosulfate. Add one drop of Sodium Thiosulfate Standard Solution, 0.1 N (Cat. No. 323-32), for each 0.3 mg of chlorine present in a one-liter sample.

For longer storage, adjust the pH to 2 or less with concentrated (about 2 mL per liter) Sulfuric Acid (Cat. No. 979-49). Store samples at 4 °C or less. Samples preserved in this manner can be stored up to 28 days. Just before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution (Cat. No. 2450-26). Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open an Ammonia Nitrogen Standard Solution, 10-mg/L as NH_3–N.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample.Use the TenSette® Pipet to add 0.2 mL, 0.4 mL and 0.6 mL of standard, respectively to the cylinders and mix each thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 0.20 mg/L ammonia nitrogen standard as follows:

1. Diluting 2.00 mL of the Ammonia Nitrogen Standard Solution, 10-mg/L, to 100 mL with deionized water. Or, use the TenSette® Pipet to prepare a 0.20 mg/L ammonia nitrogen standard by diluting 0.4 mL of a Ammonia Nitrogen Voluette® Standard Solution, 50-mg/L as NH_3–N, to 100 mL with deionized water.
2. To adjust the calibration curve using the reading obtained with the 0.20-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.30 mg/L NH_3–N

Program	95% Confidence Limits of Distribution
385	0.25–0.35mg/L NH_3–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.004 mg/L NH_3–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution. Test results are measured at 655 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Ammonia Nitrogen Reagent Set, for 10-mL samples (100 tests)			26680-00
Includes:			
(2) Ammonia Cyanurate Reagent Powder Pillows	2	100/pkg	26531-99
(2) Ammonia Salicylate Reagent Powder Pillows	2	100/pkg	26532-99

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Ammonia Nitrogen Standard Solution, 10-mg/L as NH_3–N		500 mL	153-49
Ammonia Nitrogen Standard, 2-mL PourRite® Ampule, 50-mg/L as NH_3–N		20/pkg	14791-20
Water, deionized		4 liters	272-56

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Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 10031 **Salicylate Method**

Test 'N Tube™ Vials **HR (0.4 to 50.0 mg/L NH_3–N)**

Scope and Application: For water, wastewater, and seawater

Tips and Techniques

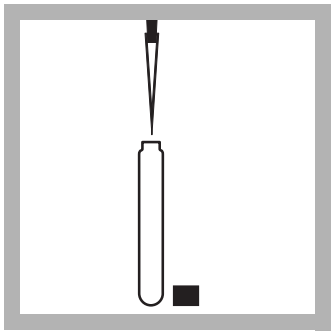
- Small sample sizes (such as 0.1 mL) may not be representative of the entire sample. Mix the sample well before testing or repeat the test, sampling from different portions of the sample.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See *Section 4* on page *55* for further information in proper disposal of these materials.
- A green color will develop if ammonia is present.

Test 'N Tube Method 10031

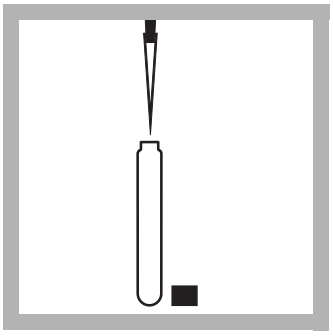
1. Touch **Hach Programs**.

Select program **343 N, Ammonia HR TNT**.

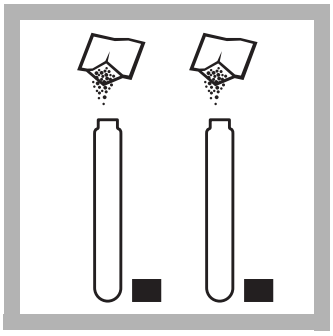
Touch **Start**.



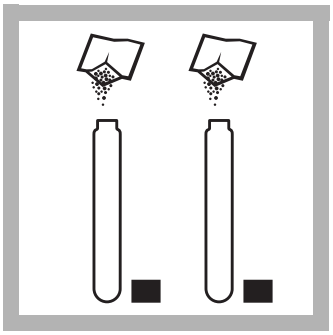
2. Add 0.1 mL of sample to one AmVer™ Diluent Reagent Test 'N Tube for High Range Ammonia Nitrogen (this is the prepared sample).



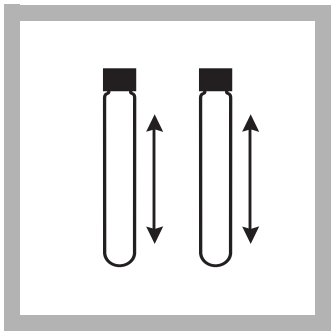
3. Add 0.1 mL of ammonia-free water to one AmVer™ Diluent Reagent Test 'N Tube for High Range Ammonia Nitrogen (this is the blank).



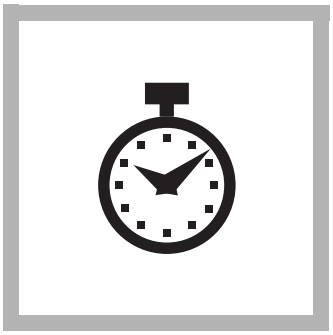
4. Add the contents of one Ammonia Salicylate Reagent Powder Pillow for 5 mL sample to each vial.



5. Add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each vial.



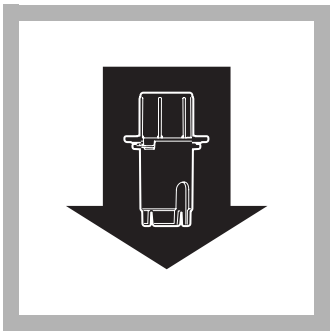
6. Cap the vials tightly and shake thoroughly to dissolve the powder.



7. Touch the timer icon.

Touch **OK**.

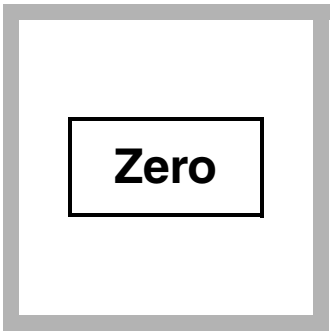
A 20-minute reaction period will begin.



8. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

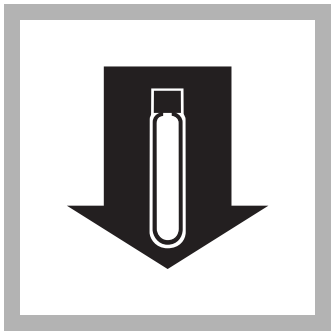
Wipe the blank and place it into the adapter



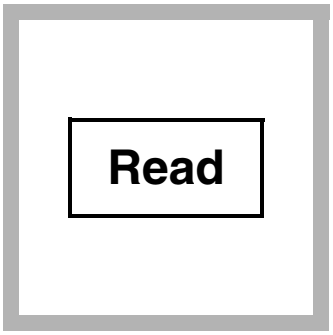
9. Touch **Zero**.

The display will show:

0.0 mg/L NH_3–N



10. Wipe the sample vial and place it into the adapter.



11. Touch **Read.**

Results will appear in mg/L NH_3–N.

Interferences

In some lab environments, airborne cross contamination of the blank is possible. Complete preparation of the blank before opening or handling any samples or standards to avoid transfer of ammonia. If sample or standard containers have already been open, move to a separate area of the lab to prepare the blank.

Interfering Substance	Interference Levels and Treatments
Acidic or basic samples	Adjust to approximately pH 7. Use 1 N Sodium Hydroxide Standard Solution (Cat. No. 1045-32) for acidic samples and 1 N Hydrochloric Acid Standard Solution (Cat. No. 134-49) for basic samples.
Calcium	50,000 mg/L as $CaCO_3$
Glycine, hydrazine	Will cause intensified colors in the prepared sample.
Magnesium	300,000 mg/L as $CaCO_3$
Iron	Eliminate iron interference as follows: **1.** Determine the amount of iron present in the sample using one of the total iron procedures. **2.** Add the same iron concentration to the deionized water in step 4. The interference will then be successfully blanked out.
Nitrite	600 mg/L as NO_2^-–N
Nitrate	5000 mg/L as NO_3^-–N
Orthophos-phate	5000 mg/L as PO_4^{3-}–P
Sulfate	6000 mg/L as SO_4^{2-}
Sulfide	Sulfide will intensify the color. Eliminate sulfide interference as follows: **1.** Measure about 350 mL of sample in a 500-mL Erlenmeyer flask. **2.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow (Cat. No. 2418-99). Swirl to mix. Filter the sample through folded filter paper (Cat. No. 692-57). Use the solution in step 4.
Turbidity and color	Give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set (Cat. No. 22653-00).

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N Sodium Thiosulfate (Cat. No. 323-32) for each 0.3 mg/L Cl_2 in a one liter sample. Preserve the sample by reducing the pH to 2 or less with at least 2 mL of Hydrochloric Acid (Cat. No. 134-49). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature. Neutralize to a pH of 7.0 with 5.0 N Sodium Hydroxide (Cat. No. 2450-26) before analysis. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Nitrogen, Ammonia PourRite® Ampule Standard, 150-mg/L NH_3–N (Cat. No. 21284-20).
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.2, 0.4 mL and 0.6 mL of standard, respectively, to each sample and mix each thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. To check accuracy, use the 10-mg/L Nitrogen, Ammonia Standard Solution or a 50-mg/L Nitrogen, Ammonia Voluette® Ampule Standard. Alternatively, prepare a 40.0-mg/L ammonia nitrogen standard solution by pipetting 20.00 mL of 100-mg/L Ammonia Nitrogen standard into a 50-mL, Class A volumetric flask. Dilute to the mark with deionized water.
2. To adjust the calibration curve using the reading obtained with the 40.0 mg/L nitrogen ammonia standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 10.0 mg/L NH_3–N

Program	95% Confidence Limits of Distribution
343	8.9–11.1 mg/L NH_3–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.4 mg/L NH_3–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a green-colored solution. Test results are measured at 655 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Reagent Set, High Range Test 'N Tube™ AmVer™ Nitrogen Ammonia	1	50 tests	26069-45

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Funnel, micro (for adding reagent)		each	25843-35
Pipet, TenSette®, 0.1 to 1.0 mL		each	19700-01
Pipet Tips, for TenSette® Pipet 19700-01		50/pkg	21856-96

Required Standards

Nitrogen Ammonia Standard Solution, 10-mg/L NH_3–N		500 mL	153-49
Nitrogen Ammonia Standard Solution, 100-mg/L NH_3–N		500 mL	24065-49
Nitrogen Ammonia Standard Solution, 150-mg/L NH_3–N, 10-mL PourRite® Ampules		16/pkg	21284-10
Nitrogen Ammonia Standard Solution, 50-mg/L NH_3–N, 10-mL Voluette® Ampules		16/pkg	14791-10
Water, deionized		4 L	272-56

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FAX: (970) 669-2932

3/04 1ed

Nitrogen, Ammonia

Method 10023 — **Salicylate Method***

Test 'N Tube™ Vials — **LR (0.02 to 2.50 mg/L NH_3–N)**

Scope and Application: For water, wastewater, and seawater

* Adapted from *Clin. Chim. Acta*, 14, 403 (1966)

Tips and Techniques

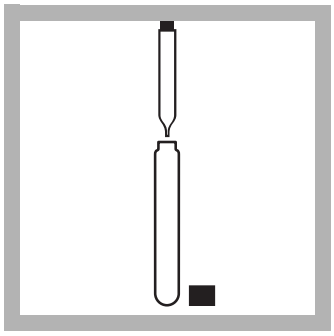
- The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See *Section 3* for further information in proper disposal of these materials.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- A green color will develop if ammonia is present.

Test 'N Tube — Method 10023

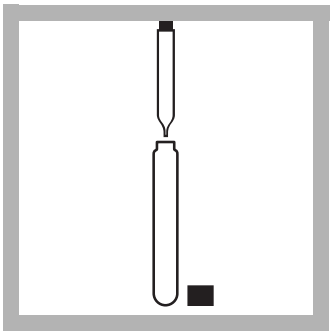
1. Touch **Hach Programs**.

Select program **342 N, Ammonia LR TNT**.

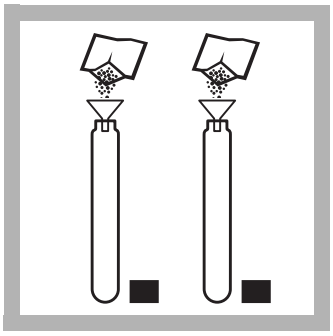
Touch **Start**.



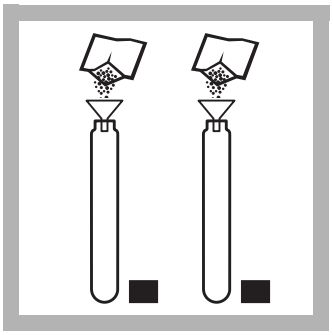
2. Add 2.0 mL of sample to one AmVer™ Diluent Reagent Test 'N Tube™ for Low Range Ammonia Nitrogen (this is the prepared sample).



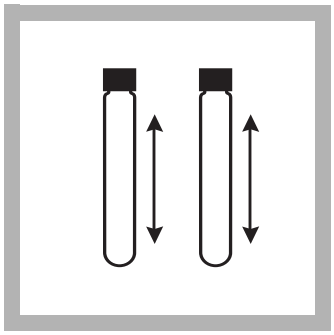
3. Add 2.0 mL of ammonia-free water to another AmVer™ Diluent Reagent Test 'N Tube for Low Range Ammonia Nitrogen (this is the blank).



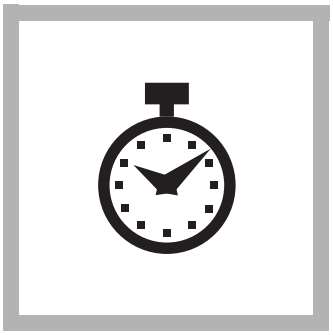
4. Add the contents of one Ammonia Salicylate Reagent Powder Pillow to each vial.



5. Add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each vial.



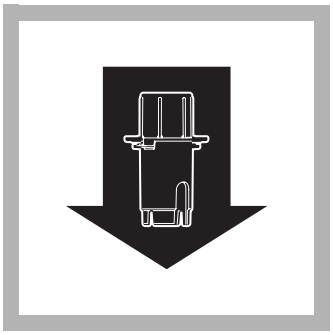
6. Cap the vials tightly and shake thoroughly to dissolve the powder.



7. Touch the timer icon.

Touch **OK**.

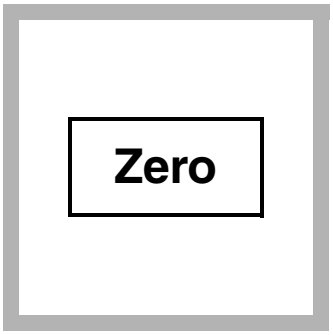
A 20-minute reaction period will begin.



8. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

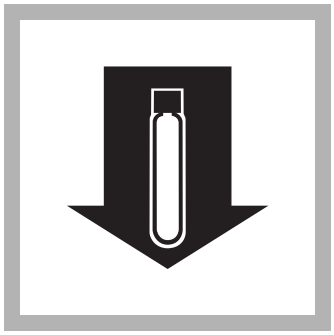
Wipe the blank and place it into the adapter.



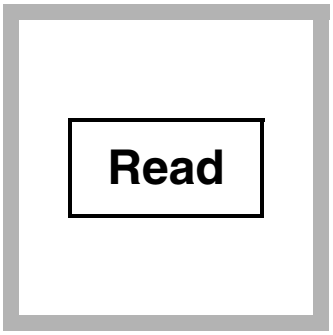
9. Touch **Zero**.

The display will show:

0.00 mg/L NH_3–N



10. Wipe the sample vial and place it into the adapter.



11. Touch **Read.**

Results will appear in mg/L NH_3–N.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	2500 mg/L as $CaCO_3$
Iron	**1.** Determine the amount of iron present in the sample by using one of the Iron, Total, procedures. **2.** Add the same iron concentration to the ammonia-free water in step 4. The interference will then be successfully blanked out.
Magnesium	15,000 mg/L as $CaCO_3$
Nitrite	30 mg/L as NO_{2-}–N
Nitrate	250 mg/L as NO_{3-}–N
Orthophos-phate	250 mg/L as PO_4^{3-}–P

(continued)

Interfering Substance	Interference Levels and Treatments
pH	Acidic or basic samples should be adjusted to approximately pH 7. Use 1 N Sodium Hydroxide Standard Solution (Cat. No. 1045-32) for acidic samples and 1 N Hydrochloric Acid Standard Solution (Cat. No. 23213-53) for basic samples.
Sulfate	300 mg/L as SO_4^{2-}
Sulfide	**1.** Measure about 350 mL of sample in a 500-mL Erlenmeyer flask. **2.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow (Cat. No. 2418-99). Swirl to mix. **3.** Filter the sample through a Folded Filter Paper (Cat. No. 1894-57) in a Filter Funnel (Cat. No. 1083-67). **4.** Use the filtered solution in *step 3*.
Other	Less common interferences such as **hydrazine** and **glycine** will cause intensified colors in the prepared sample. **Turbidity** and **color** will give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set (Cat. No. 22653-00).

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N Sodium Thiosulfate (Cat. No. 323-32) for each 0.3 mg/L Cl_2 in a one liter sample. Preserve the sample by reducing the pH to 2 or less with at least 2 mL of Hydrochloric Acid (Cat. No. 134-49). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Before analysis, warm samples to room temperature and neutralize to pH 7.0 with 5.0 N Sodium Hydroxide (Cat. No. 2450-26). Correct the test result for volume additions. See *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off an Ammonia Nitrogen Ampule Standard, 50-mg/L as NH_3–N.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

To check accuracy, use the Nitrogen Ammonia Standard Solution, 1.0 mg/L. Or, dilute 1 mL of 50-mg/L Nitrogen Ammonia Standard Solution to 50 mL with deionized water in a 50-mL volumetric flask.

Method Performance

Precision

Standard: 1.00 mg/L NH_3–N

Program	95% Confidence Limits of Distribution
342	0.96–1.04 mg/L NH_3–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.015 mg/L NH_3–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green solution. Test results are measured at 655 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Low Range Test 'N Tube™ Nitrogen-Ammonia AmVer™ Reagent Set		25 tests	26045-45

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Funnel, micro, poly	1	each	25843-35
Pipet, TenSette®, 1.0–10.0 mL	1	each	19700-10
Pipet Tips, for TenSette® Pipet 19700-10	varies	50/pkg	21997-96
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Test Tube Rack	1–3	each	18641-00

Required Standards

Nitrogen Ammonia Standard Solution, 1.0-mg/L NH_3–N		500 mL	1891-49
Nitrogen Ammonia Standard Solution, 10-mL Voluette® Ampule, 50-mg/L NH_3–N		16/pkg	14791-10
Water, deionized		4 L	272-56

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3/04 1ed

Nitrogen, Ammonium

Salicylate Method

(1.5 to 45.0 mg/L NH_4^+)

UniCell™ Vials

Scope and Application: For water, wastewater, surface water, swimming pool water, and process control

Tips and Techniques

- Adjust the pH of preserved samples to between pH 4–9 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results (see *Accuracy Check*).
- See *Section 3.2.4* on page *40* for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is 20 °C. Temperature will affect the color of the reaction and may give inaccurate results.
- Analyze the sample between 15 and 30 minutes after the reaction begins. The final color and final absorbance are reached after a reaction time of 15 minutes and remain constant for another 15 minutes.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

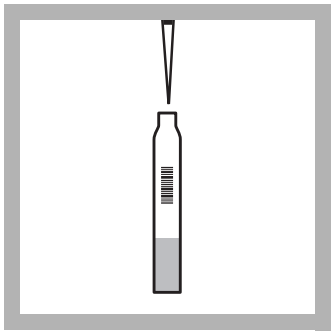
1. Touch

Hach Programs.

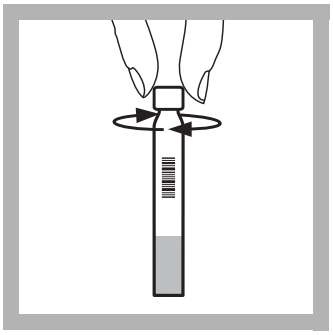
Select program

801 Ammonium, HCT 102.

Touch **Start**.

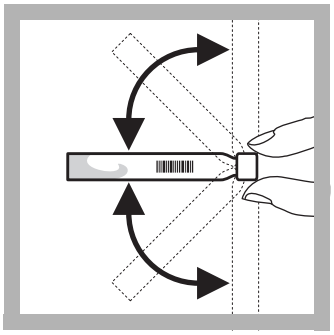


2. Add 0.2 mL of sample to one sample vial.

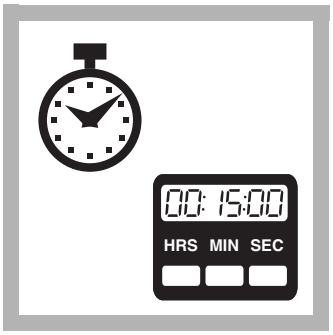


3. Immediately screw a **light green** UniCap A (HCT 100-104 A) onto the sample vial.

Close the UniCap A bottle **immediately** after use.



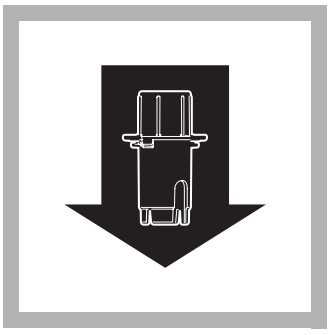
4. Invert the sample vial repeatedly until the solid reagent in the cap is dissolved.



5. Touch the timer icon.

Touch **OK**.

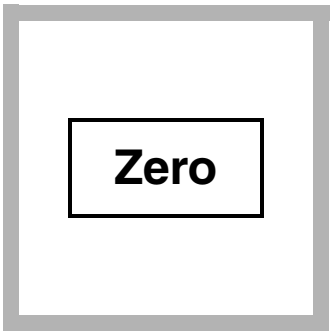
A 15-minute reaction period will begin.



6. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

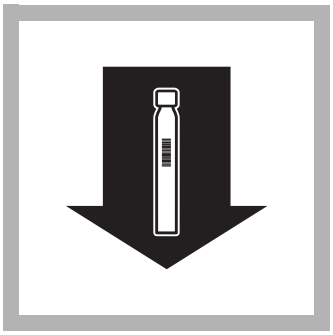
Wipe the zero (**white** cap) and sample vials and place the zero vial into the cell adapter.



7. Touch **Zero**.

The display will show:

0.0 mg/L NH_4^+
Underrange



8. When the timer beeps, place the sample vial into the cell adapter.

Touch **Read.**

Results will appear in mg/L NH_4^+.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	No interference up to:
Cl^-, SO_4^{2-}	1000 mg/L
K^+, Na^+, Ca^{2+}	500 mg/L
CO_3^{2-}, NO_3^-, Fe^{3+}, Cr^{6+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}	50 mg/L
Fe^{2+}	25 mg/L
Sn^{2+}	10 mg/L
Pb^{2+}	5 mg/L
Ag^+	2 mg/L

Sample, Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Collect samples in clean plastic or glass bottles. To preserve samples for longer periods, add 1 mL of concentrated sulfuric acid per liter and store at 4 °C.

Warm the sample to room temperature before testing. Adjust the pH to 4–9 with 5.0 N Sodium Hydroxide. Do not use mercury compounds as preservatives. Correct the test results for volume additions by dividing the total final volume (acid + base + sample) by the initial sample volume and multiplying the result by this factor. See *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Enter 1286 mg/L NH_4^+ (1000-mg/L NH_3–N) as the standard concentration.
4. Touch **OK** to accept the default values for sample volume and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Prepare three sample spikes. Fill three 100-mL mixing cylinders (Cat. No. 20886-42) with 100 mL of sample. Use a pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of (1286 mg/L NH_4^+) 1000-mg/L NH_3–N standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 25-mg/L nitrogen ammonia standard solution by pipetting 25.00 mL of 1000-mg/L Ammonia Nitrogen standard into a 1000-mL Class A volumetric flask. Dilute to the mark with deionized water.

Note: *Make sure the selected chemical form is NH_4^+–N.*

Method Performance

Precision

Standard: 25 mg/L NH_4^+–N

Program	95% Confidence Limits of Distribution
801	24.7–25.3 mg/L NH_4^+–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
801	Entire range	0.010	0.2 mg/L NH_4^+–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Ammonium ions react with hypochlorite ions and salicylate ions at pH 12.6 in the presence of sodium nitroferricyanide to form indophenol blue. Test results are measured at 694 nm.

Required Reagents

Description	Unit	Cat. No.
Ammonium - NH_4, UniCell™ HCT 102	23/pkg	HCT 102

Required Apparatus

Adapter, 16-mm Cell	each	59457-00
Pipettor, Jencons, 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00

Optional Reagents

Nitrogen Ammonia Standard Solution, 1000-mg/L NH_3–N	1 L	23541-53
Sulfuric Acid, ACS	500 mL	979-49
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	2450-26
Water, deionized	4 L	272-56

Optional Apparatus

Cylinder, mixing, 100-mL	each	20886-42
Flask, volumetric, 1000-mL	each	14574-53
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for TenSette® Pipet 19700-01	50/pkg	21856-96

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3/04 1ed

Nitrogen, Ammonium

Salicylate Method

(0.05 to 1.50 mg/L NH_4^+)

UniCell™ Vials

Scope and Application: For water, wastewater, surface water, swimming pool water, and process control

Tips and Techniques

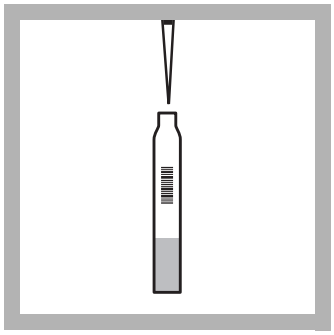
- Adjust the pH of preserved samples to between pH 4–9 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results (see *Accuracy Check*).
- See *3.2.4* on page *40* for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is 20 °C. Temperature will affect the color of the reaction and may give inaccurate results.
- Analyze the sample between 15 and 30 minutes after the reaction begins. The final color and final absorbance are reached after a reaction time of 15 minutes and remains constant for an additional 15 minutes.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

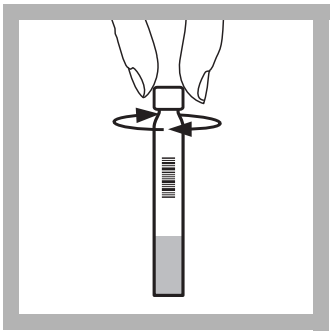
1. Touch **Hach Programs**.

Select program **802 Ammonium, HCT 100**.

Touch **Start**.

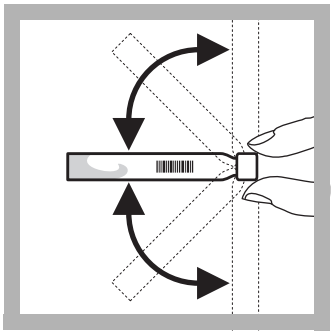


2. Add 5.0 mL of sample to a sample vial.

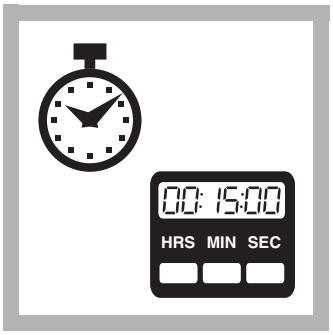


3. Immediately screw a **light green** UniCap A (HCT 100-104 A) onto the sample vial.

Close the UniCap A bottle immediately after use.



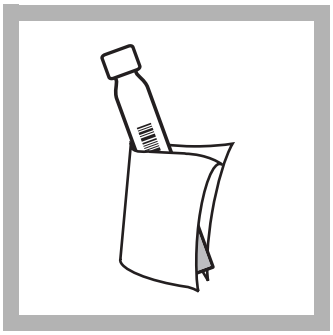
4. Invert the sample vial repeatedly until the solid reagent in the cap is dissolved.



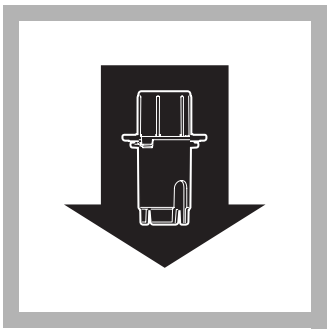
5. Touch the timer icon.

Touch **OK**.

A 15-minute reaction period will begin.



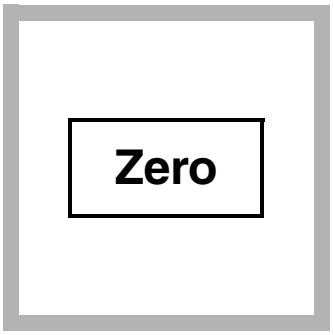
6. Wipe the zero (white cap) and sample vials.



7. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

When the timer beeps, place the zero vial into the cell adapter.



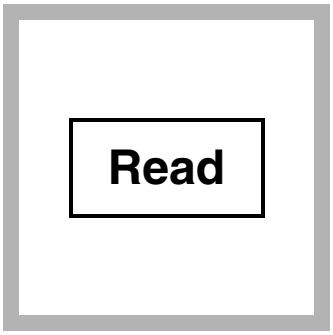
8. Touch **Zero**.

The display will show:

0.00 mg/L NH_4^+

Underrange

9. Wipe the sample vial and place it into the cell adapter.



10. Touch **Read.**

Results will appear in mg/L NH_4^+.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	No interference up to:
Cl^-, SO_4^{2-}	1000 mg/L
K^+, Na^+, Ca^{2+}	500 mg/L
CO_3^{2-}, NO_3^-, Fe^{3+}, Cr^{6+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}	50 mg/L
Fe^{2+}	25 mg/L
Sn^{2+}	10 mg/L
Pb^{2+}	5 mg/L
Ag^+	2 mg/L

Sample, Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Collect samples in clean plastic or glass bottles. To preserve samples for longer periods, add 1 mL of concentrated sulfuric acid per liter and store at 4 °C.

Warm the sample to room temperature before testing. Adjust the pH to 4–9 with 5.0 N sodium hydroxide. Do not use mercury compounds as preservatives. Correct the test results for volume additions by dividing the total final volume (acid + base + sample) by the initial sample volume and multiplying the result by this factor. See *Section 3.1.3 Correcting for Volume Additions* on page *29*

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Enter 128.6 mg/L NH_4^+ (100-mg/L NH_3–N) as the standard concentration.
4. Touch **OK** to accept the default values for sample volume and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Prepare three sample spikes. Fill three 100-mL Mixing Cylinders (Cat. No. 20886-42) with 100 mL of sample. Use a pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of (128.6 mg/L NH_4^+) 100-mg/L NH_3–N standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

To check accuracy, use the Nitrogen Ammonia Standard Solution, 1.0-mg/L. Alternatively, dilute 1 mL of 50-mg/L Nitrogen Ammonia Standard Solution to 50 mL with deionized water in a 50-mL volumetric flask.

Note: *Make sure that the selected chemical form is NH_4^+–N.*

Method Performance

Precision

Standard: 1.00 mg/L NH_4^+–N

Program	95% Confidence Limits of Distribution
802	0.76–0.80 mg/L NH_4^+–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
802	Entire range	0.010	0.01 mg/L NH_4^+–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Ammonium ions react with hypochlorite ions and salicylate ions at pH 12.6 in the presence of sodium nitroferricyanide to form indophenol blue. Test results are measured at 694 nm.

Required Reagents

Description	Unit	Cat. No.
Ammonium - NH_4, UniCell™ HCT 100	23/pkg	HCT 100

Optional Reagents

Ammonium Standard Solution, 100-mg/L as NH_3–N	500 mL	24065-49
Nitrogen Ammonia Standard, 1-mg/L as NH_3–N	500 mL	1891-49
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	2450-26
Sulfuric Acid, ACS	500 mL	979-49

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Cylinder, mixing, 100-mL	each	20886-42
Flask, volumetric, 1000-mL	each	14574-53
Pipettor, Jencons, 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00

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Nitrogen, Total

Method 10072

Persulfate Digestion Method

Test 'N Tube™ Vials

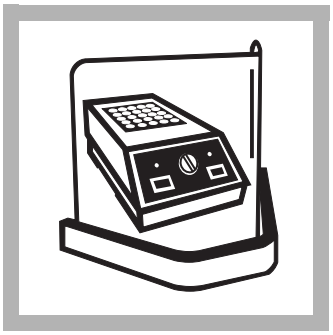
HR (10 to 150 mg/L N)

Scope and Application: For water and wastewater.

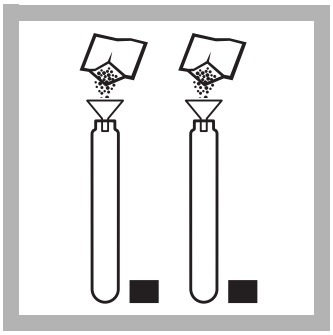
Tips and Techniques

- Digestion is required for determining total nitrogen. The digestion procedure is included below.
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- If the test overranges, repeat the digestion and measurement with diluted sample. The digestion must be repeated for accurate results.
- Use the deionized water provided in the reagent set or Organic-free Water (Cat. No. 26415-49) to prepare the standards and perform the procedure.

Test 'N Tube — Method 10072

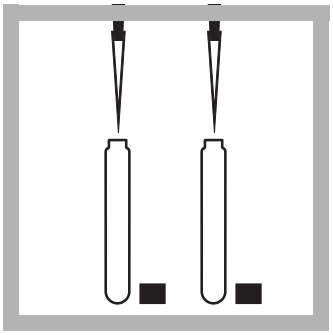


1. Turn on the COD Reactor. Heat to 103–106 °C.



2. Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two HR Total Nitrogen Hydroxide Digestion Reagent vials.

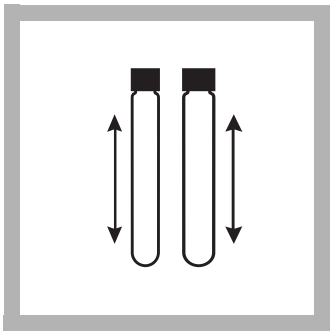
Wipe off any reagent that may get on the lid or the tube threads.



3. Add 0.5 mL of sample to a vial (this is the prepared sample).

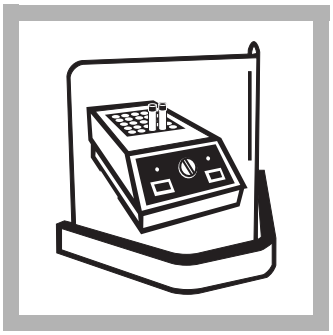
Add 0.5 mL of the deionized water included in the kit to a second vial (this is the reagent blank).

Note: *Use only water that is free of all nitrogen-containing species as a substitute for the deionized water provided.*

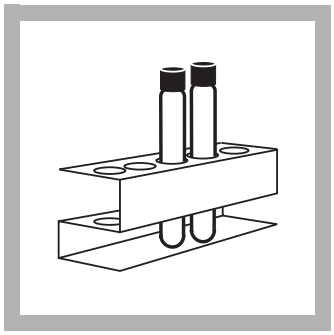


4. Cap both vials. Shake vigorously for at least 30 seconds to mix.

Note: *The persulfate reagent may not dissolve completely after shaking. This will not affect accuracy.*



5. Place the vials in the reactor. Heat for exactly 30 minutes.

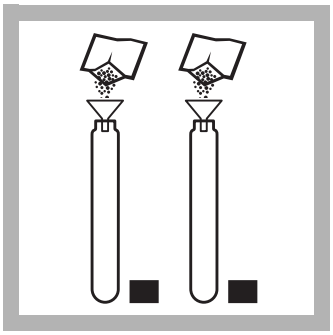


6. Using finger cots, immediately remove the hot vials from the reactor. Cool the vials to room temperature.

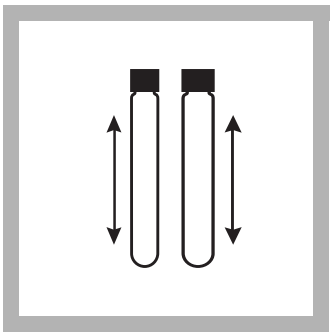
7. Touch **Hach Programs**.

Select program **395 N, Total HR TNT**.

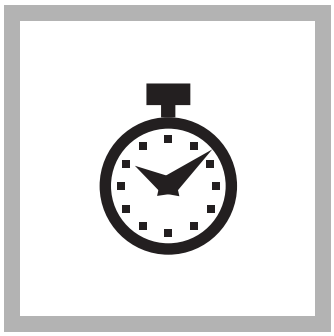
Touch **Start**.



8. Remove the caps from the digested vials and add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.



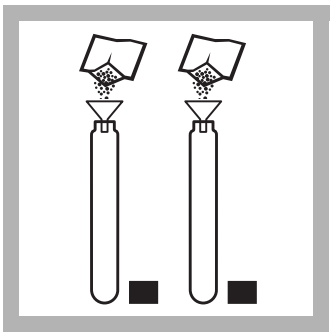
9. Cap the tubes and shake for 15 seconds.



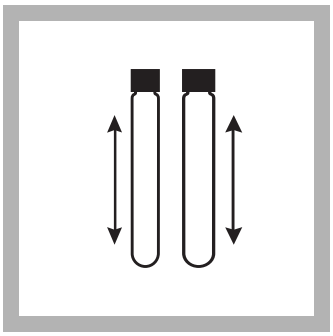
10. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.



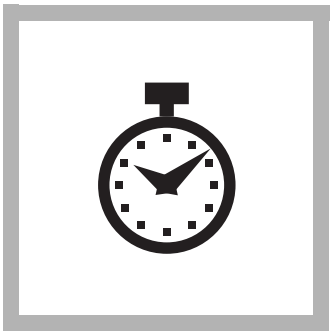
11. After the timer beeps, remove the caps from the vials and add one TN Reagent B Powder Pillow to each vial.



12. Cap the tubes and shake for 15 seconds.

Note: *The reagent will not completely dissolve. This will not affect accuracy.*

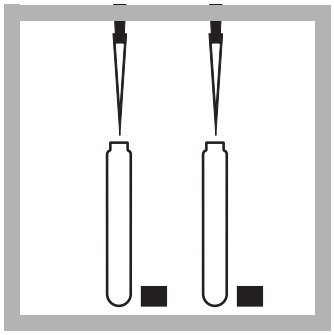
The solution will begin to turn yellow.



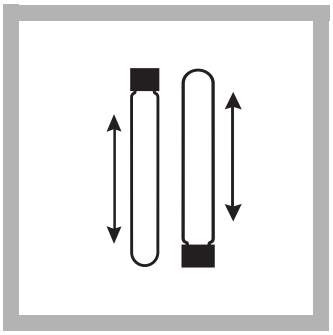
13. Touch the timer icon.

Touch **OK**.

A two-minute reaction period will begin.

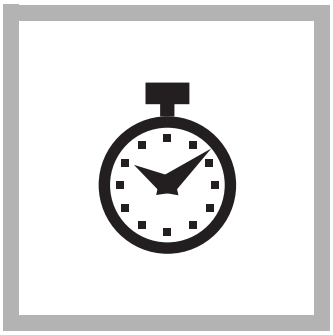


14. After the timer beeps, remove the caps from two TN Reagent C vials and add 2 mL of digested, treated sample to one vial. Add 2 mL of digested, treated reagent blank to the second TN Reagent C vial.



15. Cap the vials and invert ten times to mix. Use slow, deliberate inversions for complete recovery.

The tubes will be warm to the touch.

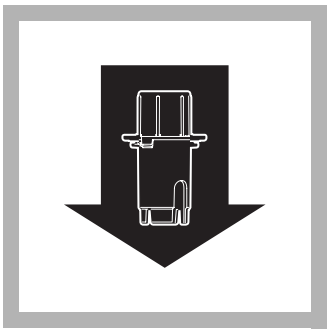


16. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

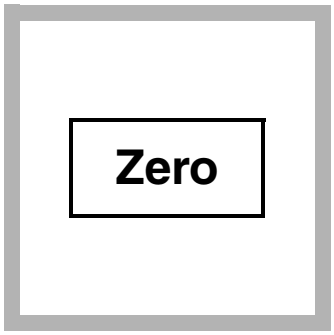
The yellow color will intensify.



17. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

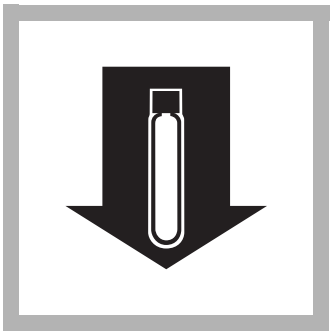
Wipe the reagent blank and place it into the adapter.



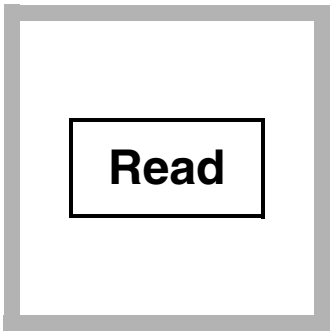
18. Touch **Zero**.

The display will show:

0 mg/L N



19. Wipe the reagent vial and place it into the adapter.



20. Touch **Read.**

Results will appear in mg/L N.

Blanks for Colorimetric Measurement

The reagent blank may be used repeatedly for measurements using the same lots of reagents. Store the reagent blank in the dark at room temperature (18–25 °C) for a maximum of seven days. If a small amount of white floc appears prior to the end of one week, discard the reagent blank and prepare a new one.

Interferences

The substances in *Table 1* have been tested and found not to interfere up to the indicated levels (in mg/L). Interfering substances that resulted in a concentration change of ±10% appear in *Table 2*.

Table 1 Non-interfering Substances

Substance	Maximum Level Tested
Barium	10.4 mg/L
Calcium	1200 mg/L
Chromium ($^{3+}$)	2 mg/L
Iron	8 mg/L
Lead	26.4 µg/L
Magnesium	2000 mg/L
Organic Carbon	600 mg/L
pH	13 pH units
Phosphorus	400 mg/L
Silica	600 mg/L
Silver	3.6 mg/L
Tin	6 mg/L

Table 2 Interfering Substances

Substance	Level and Effect
Bromide	> 240 mg/L; positive interference
Chloride	> 3000 mg/L; positive interference

Hach chemists tested this chemistry on standard nitrogen solutions prepared from the following compounds and obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Urea
- Glycine

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in ≥ 95% recovery.

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate reagent. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.

Preserve the sample by reducing the pH to 2 or less with concentrated (at least 2 mL/L) Sulfuric Acid (Cat. No. 979-49). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature and neutralize with 5 N Sodium Hydroxide (Cat. No. 2450-26) before analysis. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy Hach offers a set of three Primary Standards for Kjeldahl Nitrogen (Cat. No. 22778-00).

1. Prepare one or more of the following three solutions. Each preparation is for an equivalent 120-mg/L N standard. Use the deionized water included in the kit or water that is free of all organic and nitrogen-containing species.
   a. Weigh 1.6208 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   b. Weigh 2.1179 g of Glycine p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   c. Weigh 2.5295 g of Nicotinic p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
2. Analyze each of these solutions using the test procedure above. Calculate the percent recovery for each using this formula:

$$\% \text{ recovery} = \frac{\text{measured concentration}}{120} \times 100$$

The percent recovery should be:

Table 3

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Hach analysts have found Ammonia-PTSA to be the most difficult to digest. Other compounds may yield different percent recoveries.

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of an Ammonia Nitrogen Standard Solution, 1000-mg/L as NH_3–N, respectively, to each sample and mix thoroughly.
5. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery. The nitrogen concentration should increase by approximately 4 mg/L N for each 0.1 mL of standard added.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. For proof of accuracy, substitute 0.5 mL of a 100-mg/L ammonia nitrogen standard solution for the sample in the procedure.
2. To adjust the calibration curve using the reading obtained with a 100-mg/L N standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration. Touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 100 mg/L NH_3–N

Program	95% Confidence Limits of Distribution
395	94–106 mg/L N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	2 mg/L N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Test 'N Tube™ HR Total Nitrogen Reagent Set		50 vials	27141-00

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
COD Reactor, 115/230 VAC, North American Plug	1	each	45600-00
COD Reactor, 230 VAC, European Plug	1	each	45600-02
Funnel, micro	1	each	25843-35
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96
Safety Shield	1	each	50030-00
Test Tube Cooling Rack	1–3	each	18641-00

Required Standards

Ammonia Nitrogen Standard Sol., 1000-mg/L NH_3–N		1 liter	23541-53
Primary Standard Set, for Kjeldahl Nitrogen		set of 3	22778-00
Ammonia Nitrogen Standard Solution, 100-mg/L NH_3–N		500mL	24065-49
Water, deionized		500 mL	272-49
or Water, organic-free		500 mL	26415-49

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Nitrogen, Total

Method 10071

Persulfate Digestion Method

Test 'N Tube™ Vials

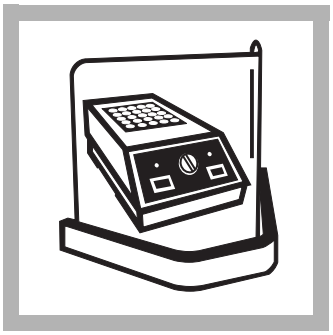
LR (0.5 to 25.0 mg/L N)

Scope and Application: For water and wastewater

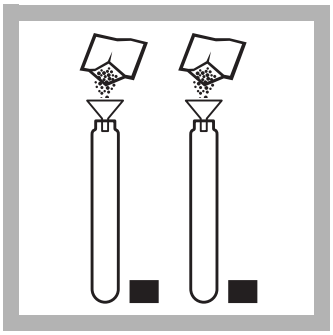
Tips and Techniques

- Digestion is required for determining total nitrogen. The digestion procedure is included below.
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- If the test overranges, repeat the digestion and measurement with diluted sample. The digestion must be repeated for accurate results.
- Use the deionized water provided in the reagent set or Organic-free Water (Cat. No. 26415-49) to prepare the standards and perform the procedure.

Test 'N Tube — Method 10071

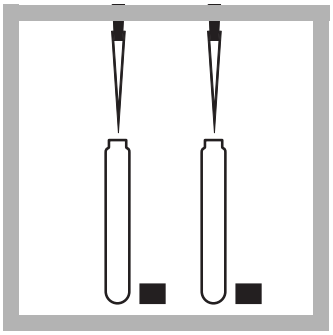


1. Turn on the COD Reactor. Heat to 103-106 °C (optimal temperature is 105 °C).



2. Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two Total Nitrogen Hydroxide Reagent vials.

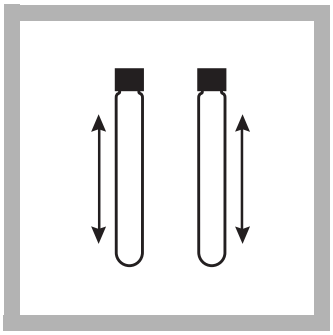
Wipe off any reagent that may get on the lid or the tube threads.



3. Add 2 mL of sample to a vial (this is the prepared sample).

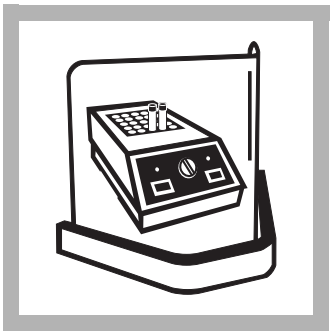
Add 2 mL of the deionized water included in the kit to a second vial (this is the reagent blank).

Note: *Use only water that is free of all nitrogen-containing species as a substitute for the deionized water provided.*

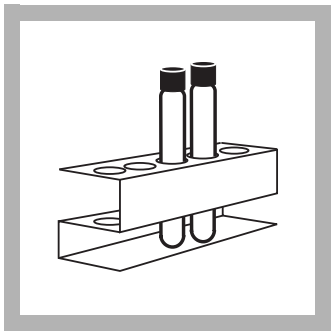


4. Cap both vials. Shake vigorously for at least 30 seconds to mix.

Note: *The persulfate reagent may not dissolve completely after shaking. This will not affect accuracy.*



5. Place the vials in the reactor. Heat for exactly 30 minutes.



6. Using finger cots, immediately remove the hot vials from the reactor. Cool the vials to room temperature.

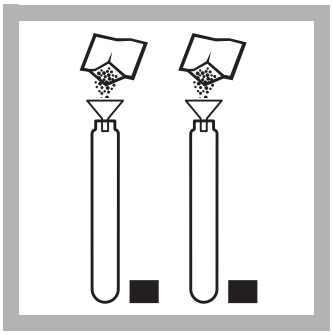
7. Touch

Hach Programs.

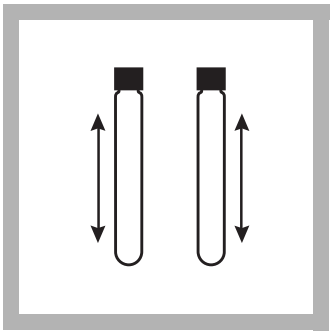
Select program

350 N, Total TNT.

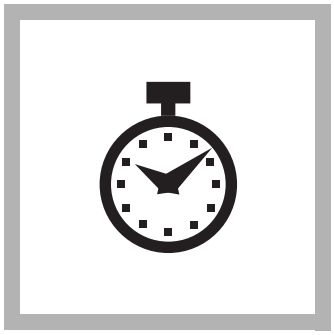
Touch **Start**.



8. Remove the caps from the digested vials and add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.



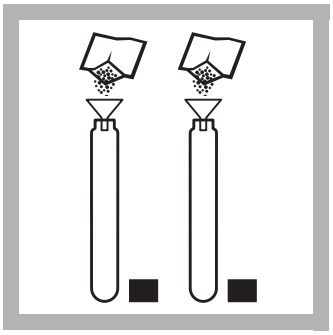
9. Cap the tubes and shake for 15 seconds.



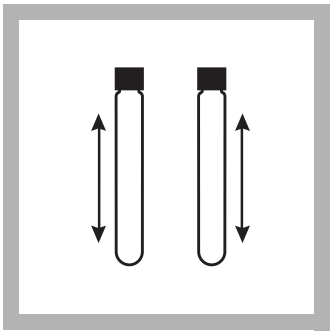
10. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.



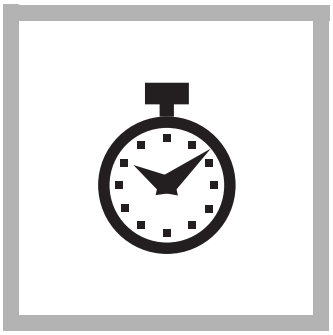
11. After the timer beeps, remove the caps from the vials and add one TN Reagent B Powder Pillow to each vial.



12. Cap the tubes and shake for 15 seconds.

Note: *The reagent may not dissolve completely after shaking. This will not affect accuracy.*

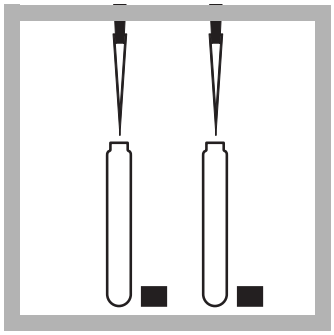
The solution will begin to turn yellow.



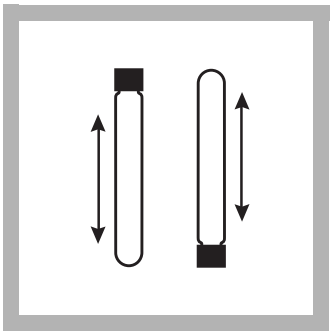
13. Touch the timer icon.

Touch **OK**.

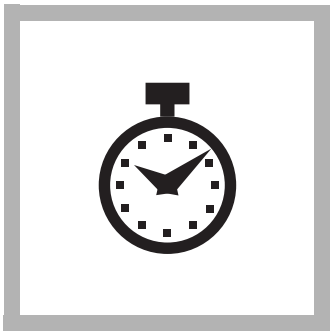
A two-minute reaction period will begin.



14. After the timer beeps, remove the caps from two TN Reagent C vials and add 2 mL of digested, treated sample to one vial. Add 2 mL of digested, treated reagent blank to the second TN Reagent C vial.



15. Cap the vials and invert ten times to mix. Use slow, deliberate inversions for complete recovery. The tubes will be warm.

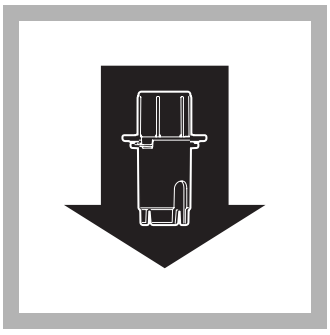


16. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

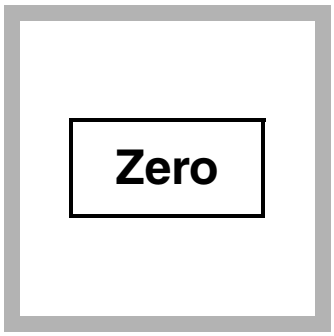
The yellow color will intensify.



17. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

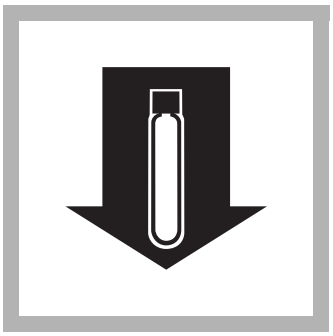
Wipe the reagent blank and place it into the adapter.



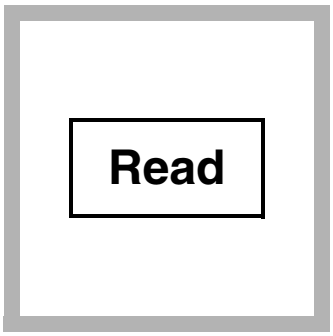
18. Touch **Zero**.

The display will show:

0.0 mg/L N



19. Wipe the reagent vial and place it into the adapter.



20. Touch **Read.**

Results will appear in mg/L N.

Blanks for Colorimetric Measurement

The reagent blank may be used up to seven days for measurements using the same lots of reagents. Store it in the dark at room temperature (18–25 °C). If a small amount of white floc appears prior to the end of one week, discard the reagent blank and prepare a new one.

Interferences

The substances in *Table 1* have been tested and found not to interfere up to the indicated levels (in mg/L). Interfering substances the resulted in a concentration change of ±10% appear in *Table 2*.

Table 1 Non-interfering Substances

Substance	Maximum Level Tested
Barium	2.6 mg/L
Calcium	300 mg/L
Chromium ($^{3+}$)	0.5 mg/L
Iron	2 mg/L
Lead	6.6 µg/L
Magnesium	500 mg/L
Organic Carbon	150 mg/L
pH	13 pH units
Phosphorus	100 mg/L
Silica	150 mg/L
Silver	0.9 mg/L
Tin	1.5 mg/L

Table 2 Interfering Substances

Substance	Level and Effect
Bromide	> 60 mg/L; positive interference
Chloride	> 1000 mg/L; positive interference

Hach chemists tested this chemistry on standard nitrogen solutions prepared from the following compounds and obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Urea
- Glycine

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in ≥ 95% recovery.

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate present. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.

Preserve the sample by reducing the pH to 2 or less with at least 2 mL of Concentrated Sulfuric Acid (Cat. No. 979-49). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature and neutralize with 5 N Sodium Hydroxide (Cat. No. 2450-32) before analysis. Correct the test result for volume additions. See *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy Hach offers a set of three Primary Standards for Kjeldahl Nitrogen (Cat. No.22778-00). Use the deionized water included in the kit or water that is free of all organic and nitrogen-containing species.

1. Prepare one or more of the following three solutions. Each preparation is for an equivalent 25 mg/L N standard.
   a. Weigh 0.3379 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   b. Weigh 0.4416 g of Glycine p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   c. Weigh 0.5274 g of Nicotinic p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
2. Analyze each of these solutions using the test procedure above. Calculate the percent recovery for each using this formula:

$$\%\ \text{recovery} = \frac{\text{measured concentration}}{25} \times 100$$

The percent recovery values are shown in *Table 3*:

Table 3

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Hach analysts have found Ammonia-PTSA to be the most difficult to digest. Other compounds may yield different percent recoveries.

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off an Ammonia Nitrogen Voluette® Ampule Standard Solution, 1000-mg/L as NH_3–N.
5. Prepare three sample spikes. Fill three mixing cylinders with 50 mL of sample. Use the TenSette® Pipet to add 0.1, 0.2, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Add 2 mL of each prepared solution, respectively, to three Total Nitrogen Hydroxide Reagent Vials.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery. The nitrogen concentration should increase by approximately 2, 4, and 6 mg/L N, respectively.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. For proof of accuracy, substitute 2 mL of a 10-mg/L ammonia nitrogen standard solution for the sample in the procedure. A single analyst should obtain less than 5% variation on replicates.
2. To adjust the calibration curve using the reading obtained with the 10-mg/L N standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40*, for more information.

Method Performance

Precision

Standard: 10 mg/L NH_3–N

Program	95% Confidence Limits of Distribution
350	9.0–11.0 mg/L N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.5 mg/L N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Test 'N Tube™ Total Nitrogen Reagent Set		50 vials	26722-45

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
COD Reactor, 115/230 VAC, North American Plug	1	each	45600-00
COD Reactor, 230 VAC, European Plug	1	each	45600-02
Funnel, micro	1	each	25843-35
Safety Shield, laboratory bench, 38 x 40 cm	1	each	50030-00
Test Tube Cooling Rack	1–3	each	18641-00

Required Standards

Ammonia Nitrogen Standard Solution as N, 100-mg/L		500 mL	24065-49
Ammonia Nitrogen Standard Sol., 10-mL Voluette® Ampule, 160-mg/L NH_3–N		16/pkg	21091-10
Primary Standard Set, Kjeldahl Nitrogen, 59-mL SCDB		3/set	22778-00
Water, deionized		500 mL	272-49
or			
Water, organic-free		500 mL	26415-49

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Nitrogen, Total

Persulfate Digestion Method

(5.0 to 40.0 mg/L N (TN_b)

UniCell™ Vials

Scope and Application: For water and wastewater

Tips and Techniques

- Warm the sample to room temperature and adjust pH between pH 4–9 with 5.0 N Sodium Hydroxide Standard Solution before testing.
- Do not use mercury compounds as preservatives.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel followed with a dry one to remove fingerprints and other marks.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check on page 3*.
- See *Adjusting the Standard Curve on page 40* in the procedure manual for information on adjusting the calibration curve.

UniCell Vials

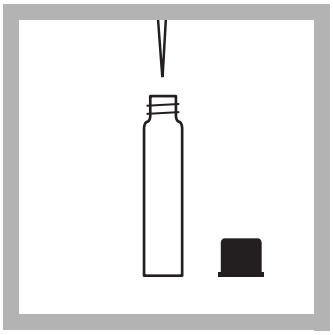
1. Touch

Hach Programs.

Select program

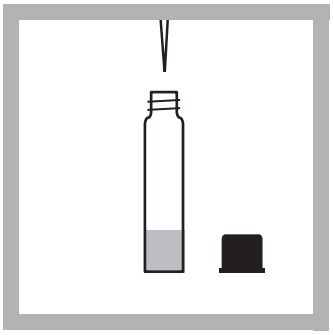
800 N, Total, HCTIII.

Touch **Start**.

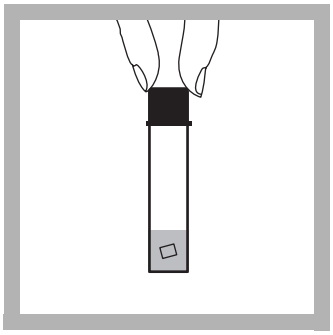


2. Pipet 0.5 mL of sample into a reaction tube (**red** cap).

Note: *Quickly complete steps 3 through 5!*

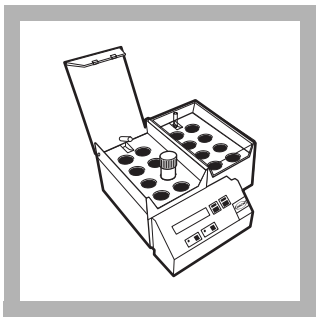


3. Immediately add 2.0 mL of Sodium Hydroxide Solution A (HCT 111 A) to the reaction tube.

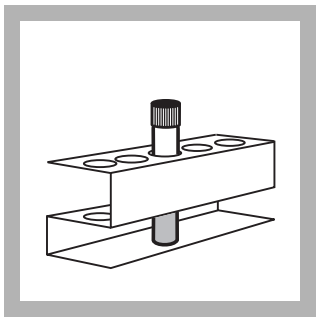


4. Immediately add 1 Oxidant Tablet B (HCT 111 B). Close the reaction tube.

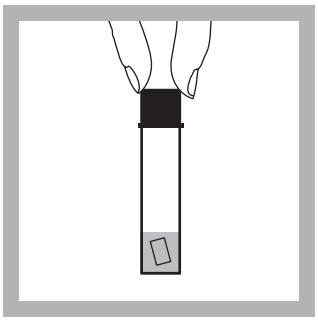
Do not invert.



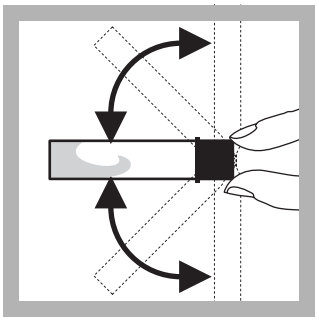
5. Heat the reaction tube in the reactor block at 100 °C for 60 minutes.



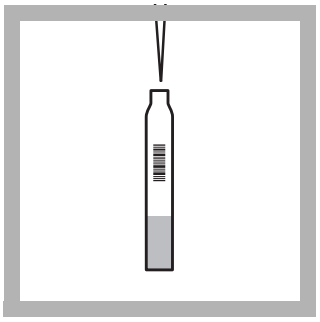
6. After the heating period, remove the tube from the reactor block and place it in a cooling rack.



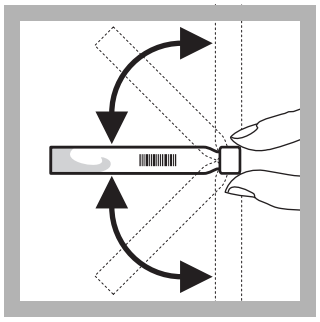
7. When the reaction tube has cooled to room temperature, add a colorless MicroCap C (HCT 111 C) and cap the tube.



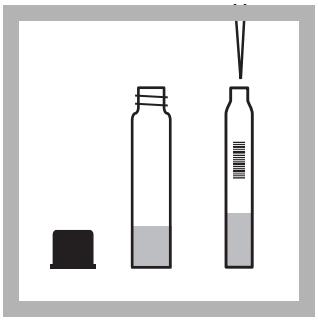
8. Invert the tube several times until the solid material in the cap is fully dissolved.



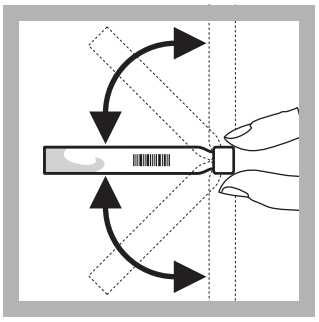
9. Pipet 0.2 mL of Dimethylphenol Solution D (HCT 111 D) into a sample vial (**blue** cap).



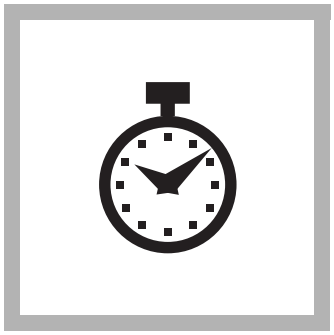
10. Cap the vial and invert three times to mix.



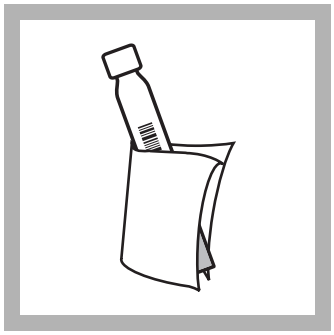
11. Immediately pipet 1.0 mL of digested sample from the reaction tube into the sample vial.



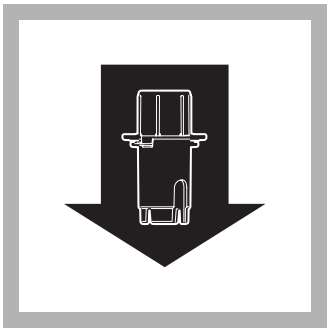
12. Cap the sample vial and invert it several times until the reagents are mixed and appear uniform.



13. Touch the timer icon. A 15-minute reaction period will begin.



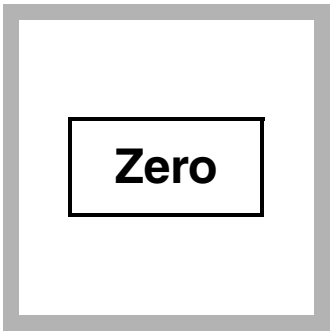
14. Wipe the outside of the zero vial (**white** cap) and the sample vial with a damp towel followed with a dry one to remove fingerprints and other marks.



15. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

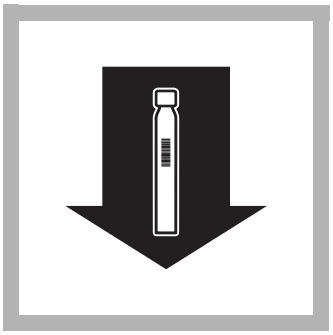
When the timer beeps, place the zero vial (**white** cap) into the cell holder.



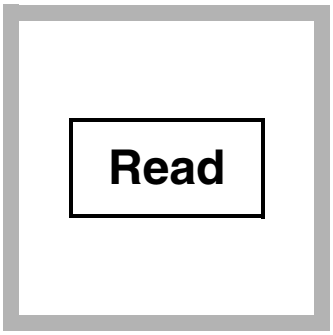
16. Touch **Zero**.

The display will show:

0.00 mg/L N



17. Wipe the prepared sample and place it into the cell holder.



18. Touch **Read.**

Results will appear in mg/L N.

Interferences

The ions listed in the table have been tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated. Ions may interfere when present in conditions exceeding those listed below:

Interfering Substance	No interference up to:
COD	1000 mg/L
Cl^-	2000 mg/L

Sample, Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Collect samples in clean plastic or glass bottles. To preserve samples for longer periods, add 1 mL of concentrated sulfuric acid per liter and store at 4 °C.

Warm the sample to room temperature before testing. Adjust the pH to 4–9 with 5.0 N Sodium Hydroxide. Do not use mercury compounds as preservatives. Correct the test results for volume additions by dividing the total final volume (acid + base + sample) by the initial sample volume and multiplying the result by this factor. See *Correcting for Volume Additions on page 29* in the procedure manual.

Accuracy Check

Standard Additions Method

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify that the units displayed are in mg/L.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Make sure that the standard concentration, sample volume, and additions volumes on the screen match your values. If they don't, touch **Edit**, then edit the values. If they match, touch **OK** to proceed. The unspiked sample reading will appear in the top row.

4. Prepare three sample spikes by filling three mixing cylinders with 50 mL of sample. Use a pipet to add 0.2, 0.4, and 0.6 mL of 1000-mg/L NO_3–N Standard, respectively, to each sample and mix thoroughly.
5. Analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery. The nitrogen concentration should increase by approximately 4, 8, and 12 mg/L N, respectively.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 20.0-mg/L NO_3–N standard solution by pipetting 2.00 mL of 1000-mg/L NO_3–N into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix.
2. To adjust the calibration curve using the reading obtained with the 20.0-mg/L N standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 20.0 mg/L NO_3–N

Program	95% Confidence Limits of Distribution
800	15.2–24.8 mg/L N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
800	Entire range	0.010	0.6 mg/L N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Bound nitrogen, both inorganically and organically bound, is oxidized to nitrate by digestion with peroxodisulphate. The nitrate ions react with 2,6-dimethylphenol in a solution of sulphuric and phosphoric acids to form a nitrophenol. Results are expressed as mg/L N or mg/L TN_b (Total Bound Nitrogen) with the same numeric value. Measurements are taken at 370 nm.

REQUIRED REAGENTS AND STANDARDS

Description	Unit	Cat. No.
Nitrogen, Total, UniCell™ HCT 111	23/pkg	HCT 111

OPTIONAL REAGENTS

Nitrogen-Nitrate (NO_3–N) Standard Solution, 1000-mg/L	500 mL	12792-49
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	2450-26
Sulfuric Acid, ACS	500 mL	979-49

OPTIONAL EQUIPMENT AND SUPPLIES

Digital Reactor Block	each	DRB 100
Flask, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 100-mL	each	20886-42
pH Paper	100/pkg	26013-00
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	400/pkg	27950-00
Reaction Tube	5/pkg	HCT 211
Test tube rack, cooling	each	18641-00

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HACH COMPANY
WORLD HEADQUARTERS
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FAX: (970) 669-2932

3/04 1ed

Nitrogen, Total Inorganic

Method 10021 — **Titanium Trichloride Reduction Method**

Test 'N Tube™ Vials — **(0.2 to 25.0 mg/L N)**

Scope and Application: For water, wastewater, and seawater

Tips and Techniques

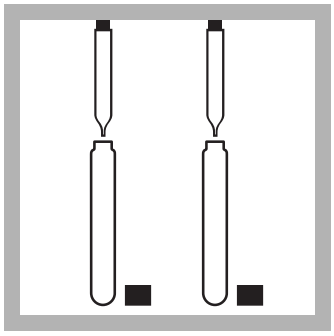
- This test requires a centrifuge.
- For safety, wear gloves while breaking ampules.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See *Section 3* on page *27* for further information on proper disposal of these materials.
- A green color will develop if inorganic nitrogen is present.

Test 'N Tube — Method 10021

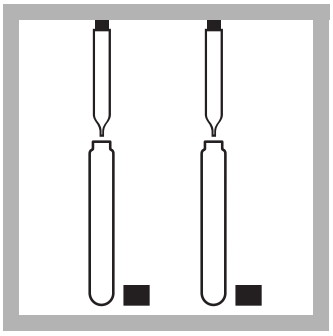
1. Touch **Hach Programs**.

Select program **346 N, Inorganic TNT**.

Touch **Start**.

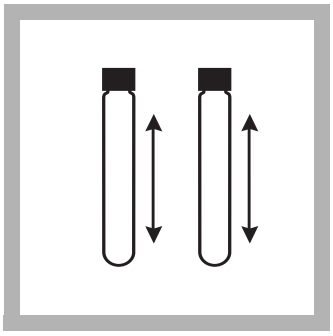


2. Pipet 1 mL of Total Inorganic Nitrogen Pretreatment Base concentrate into each of two Total Inorganic Nitrogen Pretreatment Diluent Vials.

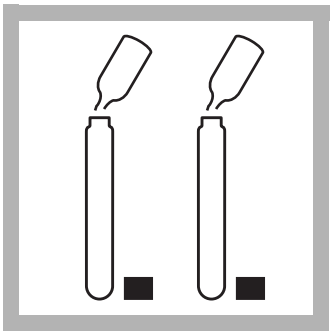


3. Pipet 1 mL of sample into one vial (this is the prepared sample).

Pipet 1 mL of deionized water into the second vial (this is the blank).



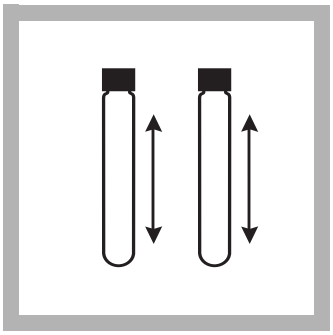
4. Cap the vials and shake for 30 seconds to mix.



5. Pour the contents of one Total Inorganic Nitrogen Reductant ampule into the vial containing the sample.

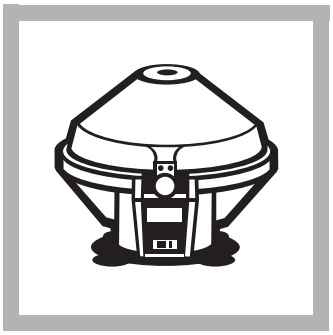
Pour the contents of another Total Inorganic Nitrogen Reductant ampule into the vial containing the blank.

A black precipitate will form immediately.



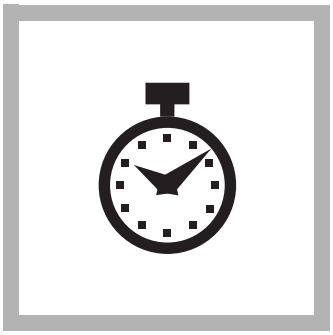
6. Cap the vials. Shake gently for 30 seconds to mix the reagents. Allow the vials to sit for at least one minute.

The precipitate should remain black after shaking. Excessive shaking will cause the precipitate to turn white and cause low results.



7. Place the vials in a centrifuge.

(If you do not have a centrifuge, wait at least 30 minutes for the solids to settle at the bottom of the vial. Proceed to *step 9*.

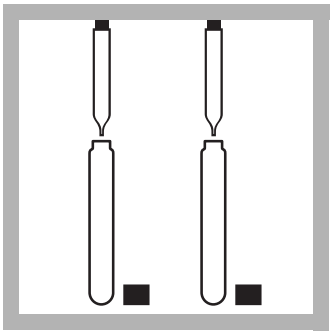


8. Touch the timer icon.

Touch **OK**.

A three-minute timer will begin.

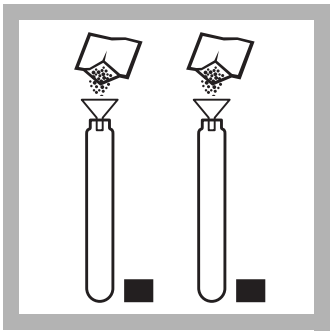
Centrifuge the vials for three minutes.



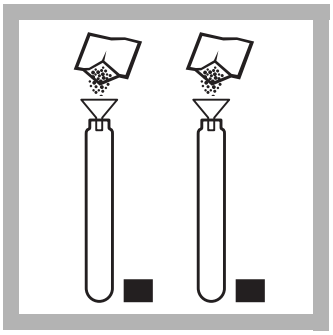
9. Using a pipet, add 2 mL of centrifuged sample to an AmVer™ Diluent Reagent Test 'N Tube™ for Low Range Ammonia Nitrogen.

Add 2 mL of centrifuged blank to another AmVer™ Dilutent Reagent Test 'N Tube™ for Low Range Ammonia Nitrogen.

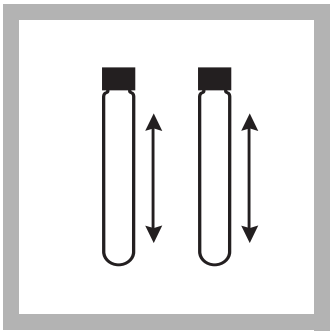
Pipet carefully to avoid disturbing the sediment.



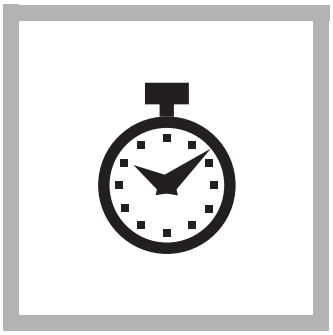
10. Using a funnel, add the contents of one Ammonia Salicylate Reagent Powder Pillow (for 5-mL samples) to each vial.



11. Using a funnel, add the contents of one Ammonia Cyanurate Reagent Powder Pillow (for 5-mL samples) to each vial.



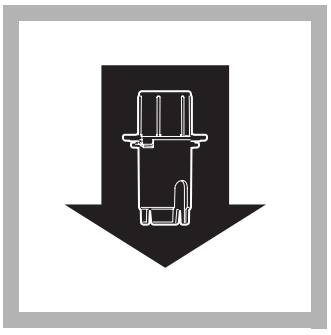
12. Cap the vials tightly and shake thoroughly to dissolve the powder.



13. Touch the timer icon.

Touch **OK**.

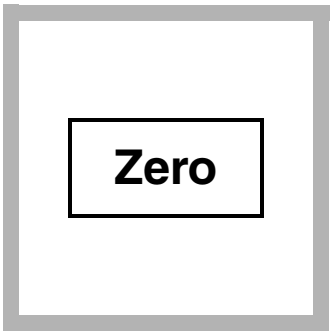
A 20-minute reaction period will begin.



14. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

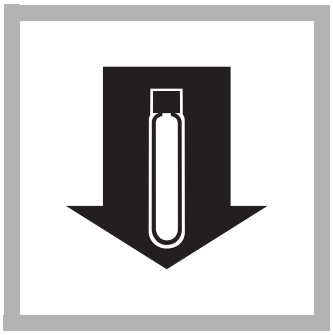
When the timer beeps, wipe the blank and place it into the adapter.



15. Touch **Zero**.

The display will show:

0.0 mg/L N



16. Wipe the prepared sample and place it into the cell holder.

Touch **Read.**

Results will appear in mg/L N.

Interferences

The following ions may interfere when present:

Interfering Substance	Interference Levels and Treatments
Calcium	Causes a positive interference at 1000 mg/L as $CaCO_3$.
Manganese (IV)	Causes a negative interference at 3 mg/L.
Magnesium	Causes a positive interference at 1000 mg/L as $CaCO_3$.
Sulfide	Causes a negative interference at 3 mg/L.
Sulfate	Causes a negative interference at 250 mg/L.

The following do not interfere below the levels listed:

Substance	Levels Tested
Al^{3+}	8 mg/L
Ba^{2+}	40 mg/L
Cu^{2+}	40 mg/L
Fe^{3+}	8 mg/L
Zn^{2+}	80 mg/L
F^-	40 mg/L
PO_4^{3-}–P	8 mg/L
SiO_2	80 mg/L
EDTA	80 mg/L

Sample Collection, Preservation, and Storage

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N Sodium Thiosulfate (Cat. No. 323-32) for each 0.3 mg/L Cl_2 in a one-liter sample. Preserve the sample by reducing the pH to 2 or less with concentrated (at least 2 mL) Hydrochloric Acid (Cat. No. 134-49). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature. Neutralize with 5 N Sodium Hydroxide (Cat. No. 2450-26) before analysis. Correct the test result for volume additions. See *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See the instrument manual for more information.
4. Snap the neck off a fresh HR Nitrate Nitrogen PourRite Ampule Standard, 500-mg/L NO_3^-–N.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

To check accuracy, use a 10.0-mg/L Nitrate Nitrogen Standard Solution. Alternatively, prepare this by diluting 1 mL of solution from a Nitrate Nitrogen Voluette® Ampule Standard, 500-mg/L NO_3^-–N, to 50 mL with deionized water. The result should be 9–10 mg/L N.

Method Performance

The total inorganic nitrogen test is designed to provide an estimate of the total nitrite, nitrate, and ammonia nitrogen load present in a water or wastewater sample. This test is most applicable to the monitoring of samples taken from an industrial process stream or a wastewater treatment stream where it is important to track the inorganic nitrogen load as it passes through the treatment process. The test does exhibit different recoveries of each of the three nitrogen species, as summarized below. The test is not recommended for use when quantifying only one of the three species. In that case, specific procedures for each particular analyte would be more appropriate.

Species Recovery

Nitrogen Form	Recovery
NH_3–N	112%
NO_3^-–N	100%
NO_2^-–N	77%

Precision

Standard: 20.0 mg/L NO_3^-–N

Program	95% Confidence Limits of Distribution
346	17.0–22.2 mg/L NO_3^-–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.2 mg/L NO_3^-–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Titanium (III) ions reduce nitrate and nitrite to ammonia in a basic environment. After centrifugation to remove solids, the ammonia is combined with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green colored solution. Test results are measured at 655 nm.

Nitrogen, Total Inorganic

Required Reagents and Standards

Description	Quantity Required Per Test	Unit	Cat. No.
Total Inorganic Nitrogen Pretreatment Reagent Set ($TiCl_3$ Reduction Method)	25 tests		26049-45
Test 'N Tube™ AmVer™ Nitrogen-Ammonia Reagent Set	25 tests		26045-45
Water, deionized	1 mL	100 mL	272-42

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Centrifuge, 115 VAC, 6 x 15 mL	1	each	26765-00
Centrifuge, 220 VAC, 6 x 15 mL	1	each	26765-02
Funnel, micro	1	each	25843-35
Pipet, TenSette®, 1.0–10.0 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	varies	50/pkg	21997-96
Pipette, volumetric, Class A, 1.00-mL	1	each	14515-35
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Test Tube Rack	1	each	18641-00

Required Standards

Nitrate Nitrogen Standard Solution, 10-mg/L NO_3^-–N	500 mL	307-49
Nitrate Nitrogen Standard Solution, 2-mL Ampule, 500-mg/L NO_3^-–N	20/pkg	14260-20
Water, deionized	4 liters	272-56

DR/2400

Nitrogen, Total Kjeldahl

Method 8075

Nessler Method* (Digestion Required)

(1 to 150 mg/L)

Scope and Application: For water, wastewater, and sludge; digestion is required.

* Adapted from Hach, et. al., *Journal of Association of Official Analytical Chemists*, 70(5) 783-787 (1987); Hach, et. al., *Journal of Agricultural and Food Chemistry*, 33(6) 1117-1123 (1985); *Standard Methods for the Examination of Water and Wastewater*

Tips and Techniques

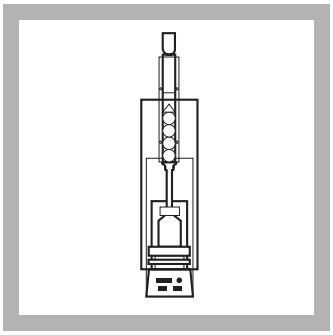
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.
- If using the Flow-Thru Cell, periodically clean the cell by pouring a few sodium thiosulfate pentahydrate crystals (Cat. No. 460-01) into the cell funnel. Flush it through the funnel and cell with enough deionized water to dissolve. Rinse out the crystals.
- Hold droppers and dropper bottles vertically, **not at an angle**, when dispensing reagent.
- Nessler reagent contains mercuric iodide. Both the sample and blank will contain mercury (D009) at concentrations regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. See *Section 4* on page *55* for more information on proper disposal of these materials.

Method 8075

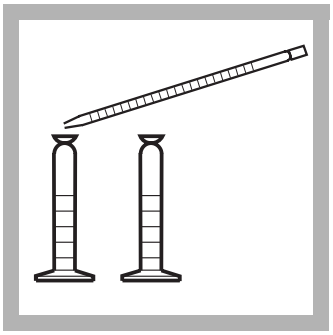
1. Touch **Hach Programs**.

Select program **399 Nitrogen, TKN**.

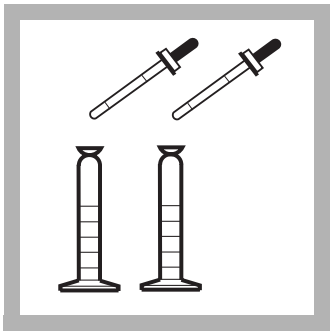
Touch **Start**.



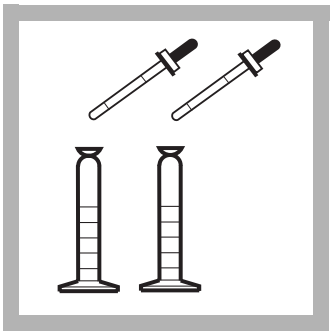
2. Digest the sample amount as described in the Digesdahl® Digestion Apparatus Instruction Manual. Digest an equal amount of deionized water as the blank.



3. Select the appropriate analysis volume of the digested sample given in *Table 1* on page *4*. Pipet the analysis volume from the sample and the blank into separate 25-mL mixing graduated cylinders.

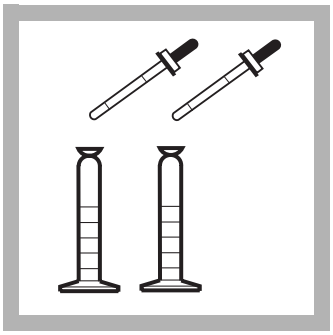


4. Add one drop of TKN Indicator to each cylinder.

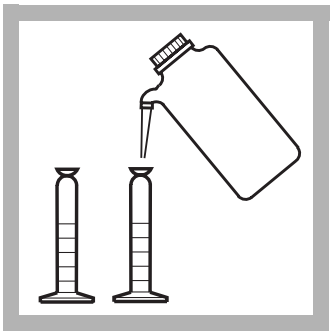


5. If the aliquot is less than 1 mL, proceed to *step 6*.

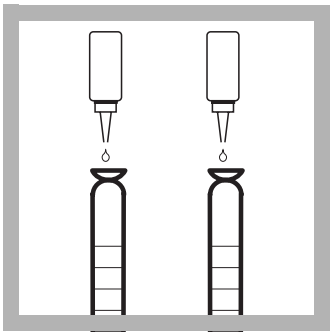
If it is greater than 1 mL, add drops of 8.0 N KOH to each cylinder until the first flash of blue color appears. Stopper and invert the cylinder after each addition. Proceed to the next step.



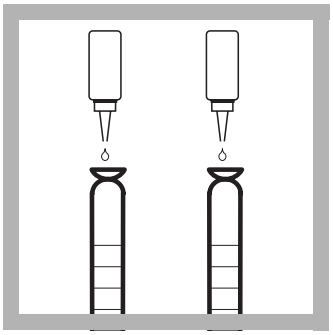
6. Add 1.0 N KOH to each cylinder, one drop at a time, mixing after each addition. Continue until the first permanent blue color appears.



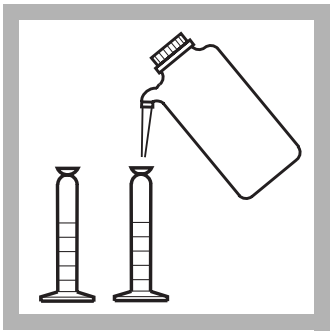
7. Fill both cylinders to the 20-mL mark with deionized water.



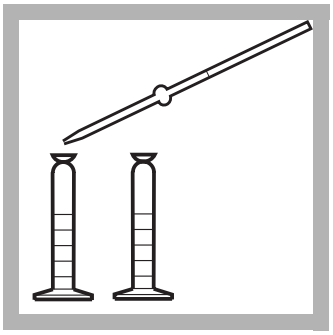
8. Add three drops of Mineral Stabilizer to each cylinder. Stopper and invert several times to mix.



9. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder. Stopper and invert several times to mix.

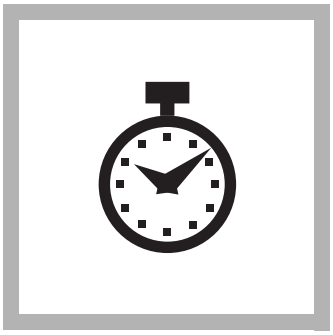


10. Fill both cylinders to the 25-mL mark with deionized water. Stopper and invert several times to mix.



11. Pipet 1.00 mL of Nesslers Reagent to each cylinder. Stopper and invert repeatedly.

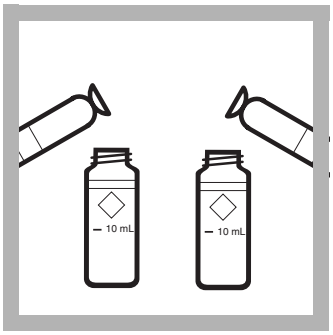
The solution should not be hazy. Any haze (turbidity) will cause incorrect results.



12. Touch the timer icon.

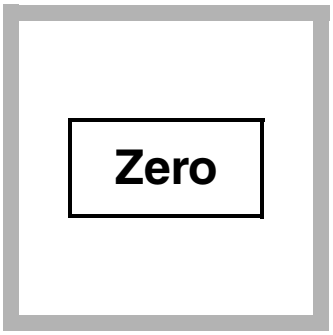
Touch **OK**.

A two-minute reaction period will begin.



13. When the timer beeps, pour the contents of each cylinder into separate 10-mL sample cells.

14. Wipe the blank and place it into the cell holder.



15. Touch **Zero**.

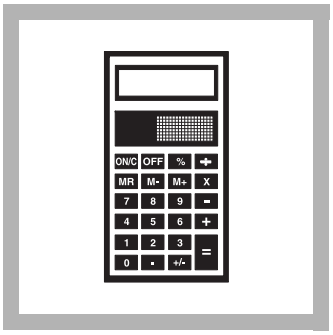
The display will show:

0 mg/L TKN

16. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L TKN.



17. Calculate sample TKN as follows:

$$\text{ppm TKN} = \frac{75 \times A}{B \times C}$$

Where:

A = mg/L read from the display

B = g (or mL of water) sample taken for digest

C = mL analysis volume of digested sample

Table 1

AQUEOUS SAMPLES (Solutions of suspensions in water—less than 1% solids)	
Expected Nitrogen Concentration (mg/L)	**Analysis Volume (mL)**
0.5–28	10.0
2–112	5.0
11–560	2.00
45–2250	1.00
425–22500	0.500
DRY SAMPLES	
Expected Nitrogen Concentration (mg/L)	**Analysis Volume (mL)**
42–2200	10.0
106–5600	5.00
350–18,000	2.00
1000–56,000	1.00
4200–220,000	0.50
OILS AND FATS	
Expected Nitrogen Concentration (mg/L)	**Analysis Volume (mL)**
85–4500	10.0
210–11,000	5.00
2100–110,000	1.00

Sample Collection, Storage, and Preservation

Collect samples in clean glass or plastic containers. Adjust the pH to 2 or less with Sulfuric Acid (Cat. No. 979-49) (about 2 mL per liter) and cool to 4 °C (39 °F). Preserved samples can be stored up to 28 days.

Accuracy Check

Kjeldahl Nitrogen Standard Method

This procedure checks digestion efficiency and indicates that amount of bound nitrogen that is freed during digestion. The methods and standards available to check digestion technique are found in the *Accuracy Check* section following the procedure in the *Digesdahl® Digestion Apparatus Instruction Manual*. Using the digested Kjeldahl standard, perform the above TKN analysis on the colorimeter. The TKN value should come within ±3% of the value of the prepared Kjeldahl standard.

Standard Solution Method (to check calibration accuracy only)

1. Add one drop of TKN Indicator to each 25-mL graduated mixing cylinder.
2. Fill one cylinder to the 20-mL mark with deionized water. Fill the other cylinder to the 20-mL mark with a 1.0-mg/L NH_3–N solution.
3. Add 3 drops of Mineral Stabilizer to each cylinder. Invert several times to mix.
4. Add 3 drops of Polyvinyl Alcohol Dispersing agent to each cylinder.
5. Perform the TKN procedure as described in *step 10* to *step 16*. The display should show 26–27 mg/L TKN.

Method Performance

Precision

Standard: 120 mg/L NH_3–N

Program	95% Confidence Limits of Distribution
399	107–133 mg/L NH_3–N

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	1 mg/L NH_3–N

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The term *Total Kjeldahl Nitrogen* refers to the combination of ammonia and organic nitrogen. However, only the organic nitrogen compounds appearing as organically bound nitrogen in the trinegative state are determined in this test. Nitrogen in this form is converted into ammonium salts by the action of sulfuric acid and hydrogen peroxide. The ammonia is then analyzed by a modified Nessler method test. Test results are measured at 460 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Nitrogen Reagent Set, 0–150 mg/L, Nesslers Method		250 tests	24953-00
Includes:			
Hydrogen Peroxide, 50%	20 mL	490 mL	21196-49
Mineral Stabilizer	6 drops	50 mL SCDB	23766-26
Nesslers Reagent	2 mL	500 mL	21194-49
Polyvinyl Alcohol Dispersing Agent	6 drops	50 mL SCDB	23765-26
Potassium Hydroxide Standard Solution, 1.0 N	varies	50 mL SCDB	23144-26
Potassium Hydroxide Standard Solution, 8.0 N	varies	100 mL MDB	282-32
Sulfuric Acid, ACS, concentrated	6 mL	500 mL	979-49
TKN Indicator Solution	2 drops	50 mL SCDB	22519-26

Required Apparatus

Boiling Chips, silicon carbide	2–3	500 g	20557-34
Cots, finger	2	2/pkg	14647-02
Cylinder, graduated mixing, 25-mL	2	each	26362-40
Select one based on available voltage:			
Digesdahl® Digestion Apparatus, 115 VAC	1	each	23130-20
Digesdahl® Digestion Apparatus, 220 VAC	1	each	23130-21
Pipet, TenSette®, 0.1–1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96
Safety Shield	1	each	50030-00
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Nitrogen Standard Solution, 1-mg/L NH_3–N		500 mL	1891-49
Nitrogen Standard Solution, Voluette® Ampule, 10-mL		16/pkg	21284-10

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Organic Carbon, Total

Method 10128

Direct Method*

HR (100 to 700 mg/L C)

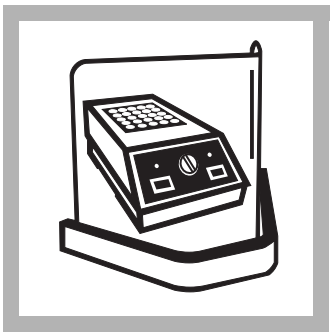
Scope and Application: For wastewater and industrial waters

* U.S. Patent 6,368,870

Tips and Techniques

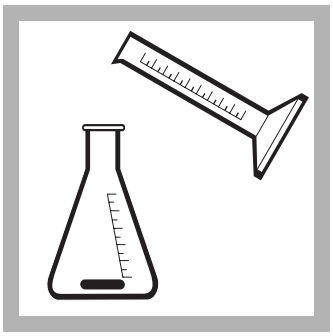
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- A reagent blank is required for each series of samples.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Method 10128

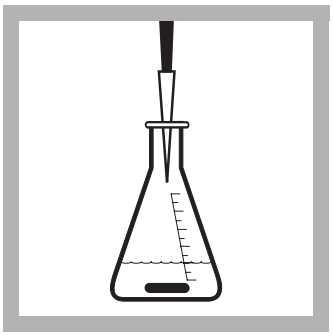


1. Turn on the COD reactor.

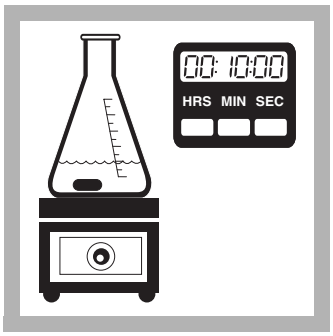
Heat to 103–105 °C.



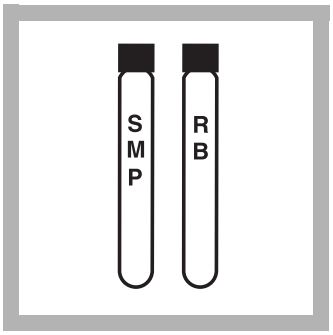
2. Use a graduated cylinder to add 10 mL of sample to a 50-mL Erlenmeyer flask that contains a stir bar.



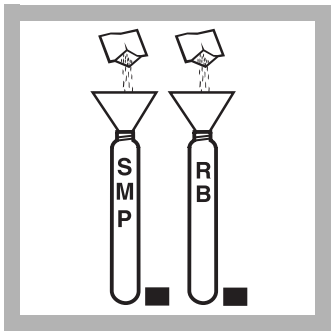
3. Add 0.4 mL of Buffer Solution, pH 2.0. Use pH paper to make sure the sample pH is 2.



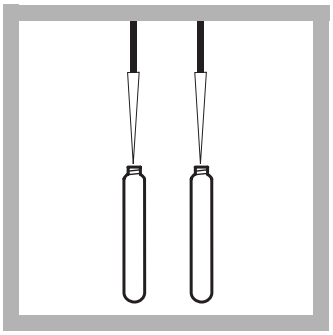
4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.



5. Label two High Range Acid Digestion vials *sample* and *reagent blank*.



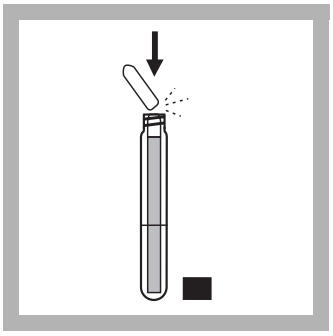
6. Use a funnel to add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).



7. Use a TenSette® Pipet to add 0.3 mL of organic-free water to the reagent blank vial and 0.3 mL of prepared sample to the sample vial. Swirl to mix.

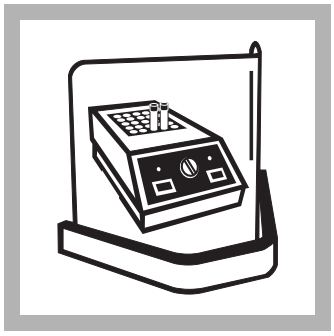
8. Rinse two blue Indicator Ampules with deionized water and wipe them with a soft, lint-free wipe.

Do not touch the ampules sides after wiping. Pick them up by the top.

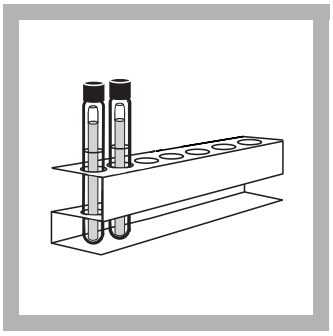


9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, snap the top off the ampule and allow it to drop into the Acid Digestion vial.

Do not invert or tilt the vial after inserting the ampule.



10. Cap the vial assemblies tightly and place them in the COD reactor for 2 hours at 103–105 °C.



11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for one hour for accurate results.

The liquid in the reagent blank vial should be dark blue.

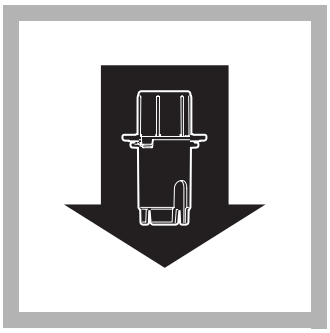
12. Touch

Hach Programs.

Select program

426 Organic Carbon HR.

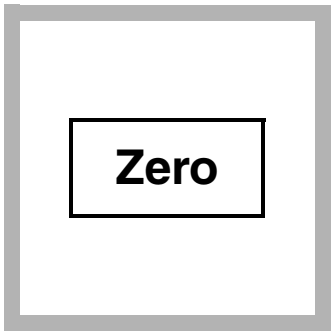
Touch **Start**.



13. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Wipe the reagent blank vial assembly and place it into the cell adapter.

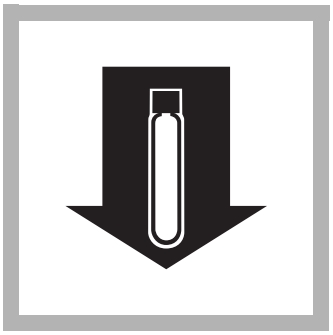


14. Touch **Zero**.

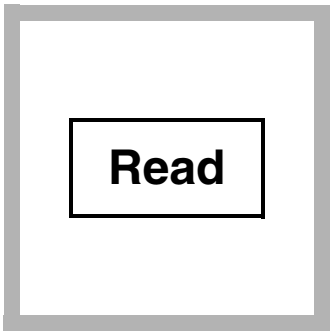
The display will show:

0 mg/L C

then **Underrange** until the sample is read.



15. Wipe the reagent vial assembly and place it into the cell adapter.



16. Touch **Read.**

Results will appear in mg/L C.

Note: *The display will show* **Underrange** *when the mg/L C value is below the lower limit of the method.*

Interferences

The following have been tested for interference and found not to interfere up to the indicated levels:

Substance	Maximum Level Tested
Aluminum	10 mg/L
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br
Bromine	25 mg/L Br_2
Calcium	2000 mg/L as $CaCO_3$
Chloride	5000 mg/L
Chlorine	10 mg/L Cl_2
Chlorine Dioxide	6 mg/L ClO_2
Copper	10 mg/L
Cyanide	10 mg/L CN
Iodide	50 mg/L
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Magnesium	2000 mg/L as $CaCO_3$
Manganese (VII)	1 mg/L
Monochloramine	14 mg/L NH_2Cl as Cl_2
Nitrite	500 mg/L NO_2^-
Ozone	2 mg/L O_3

(continued)

Substance	Maximum Level Tested
Phosphate	3390 mg/L PO_4^{3-}
Silica	100 mg/L SiO_2
Sulfate	5000 mg/L SO_4^{2-}
Sulfide	20 mg/L S^{2-}
Sulfite	50 mg/L SO_3^{2-}
Zinc	5 mg/L

If the sample contains greater than 1000 mg/L $CaCO_3$ alkalinity, lower the sample pH to less than 7 before testing by adding Sulfuric Acid Solution (Cat. No. 2449-32).

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 900 NTU have been tested without interference.

Sampling and Storage

Collect samples in clean glass bottles. Rinse the sample bottle several times with the sample to be collected. Fill the bottle completely full before capping. Test samples as soon as possible. Acid preservation is not recommended. Homogenize samples containing solids to assure representative samples.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Prepare a 300-mg/L C standard as described in Standard Solutions Method, below.
2. Use the TenSette pipet to add 0.1, 0.2, and 0.3 mL of the 300-mg/L C standard to each of three Acid Digestion vials.
3. Add the contents of one TOC Persulfate powder pillow to each vial.
4. Add 0.3 mL sample to each vial. Swirl to mix.
5. Proceed with the procedure starting at *step 8* on page *2*.
6. The mg/L C concentration should increase by 100 mg/L for each 0.1 mL increment.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

1. Prepare a 1000-mg/L organic carbon stock standard by dissolving 2.1254 g dry primary standard Potassium Acid Phthalate in Organic-Free Reagent Water and dilute to 1000 mL. This stock standard is stable for about 1 month at room temperature.

   Alternatively, open one ampule of TOC Standard Solution (Cat. No. 27915-05).
2. Prepare a 300-mg/L C standard by transferring 15.00 mL of the stock standard to a 50-mL Class A volumetric flask. Dilute to volume using Organic-Free Reagent Water. Stopper and mix thoroughly. Prepare this standard fresh daily.

Method Performance

Precision

Program	mg/L C	95% Confidence Limits of Distribution
426	100	±14 mg/L C
426	250	±13 mg/L C
426	400	±15 mg/L C
426	550	±23 mg/L C
426	700	±34 mg/L C

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Mid-range	0.010	4 mg/L C

See *Section 3.4.5 Sensitivity* on page *44* for more information.

To test for TOC levels below 100 mg/L C, use Method 10173. For more information on determining precision data and method detection limits, refer to *Section 3.4 Method Performance* on page *42*.

Summary of Method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial, organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The adsorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample. Test results are measured at 598 and 430 nm.

Organic Carbon, Total

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Total Organic Carbon Direct Method			
High Range Test 'N Tube™ Reagent Set		50 vials	27604-45
Includes:			
Acid Digestion Solution Vials, High Range TOC	1	50/pkg	*
Buffer Solution, Sulfate	0.4 mL	25 mL	452-33
Funnel, micro	1	each	25843-35
Indicator Ampules, High Range TOC	1	10/pkg	*
TOC Persulfate Powder Pillows	1	50/pkg	*
pH Paper	1	5/pkg	391-33
Water, organic-free	3.0 mL	500 mL	26415-49

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
COD Reactor, 115/230 VAC (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 VAC (Europe)	1	each	45600-02
Cylinder, graduated, 10-mL	1	each	508-38
Flask, Erlenmeyer, 50-mL	1	each	505-41
Magnetic Stirrer	1	each	23436-00
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet	2	50/pkg	21997-96
Safety Shield, laboratory bench	1	each	50030-00
Stir Bar, Magnetic	1	each	45315-00
Test Tube Rack	1–3	each	18641-00
Wipes, Disposable, Kimwipes®	1	280/pkg	20970-00

Required Standards

Potassium Acid Phthalate	500 g	315-34
TOC Standard Solution (KHP Standard, 1000-mg/L C)	5/pkg	27915-05

* Not sold separately.

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FAX: (970) 669-2932

3/04 1ed

Organic Carbon, Total

Method 10173

Direct Method*

MR (15 to 150 mg/L C)

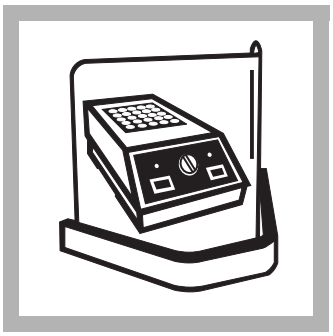
Scope and Application: For wastewater and industrial waters

* U.S. Patent 6,368,870

Tips and Techniques

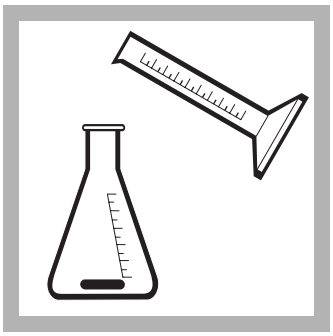
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- A reagent blank is required for each series of samples.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Method 10173



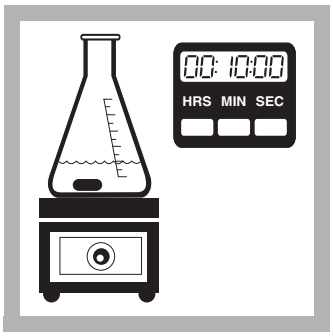
1. Turn on the COD reactor.

Heat to 103–105 °C.

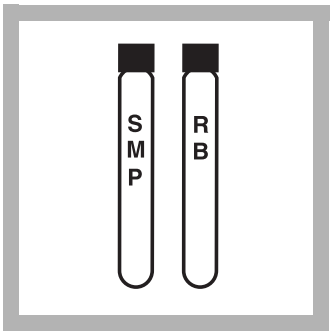


2. Use a graduated cylinder to add 10 mL of sample to a 50-mL Erlenmeyer flask that contains a stir bar.

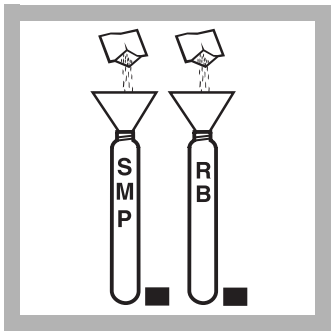
3. Add 0.4 mL of Buffer Solution, pH 2.0. Use pH paper to make sure the sample pH is 2.



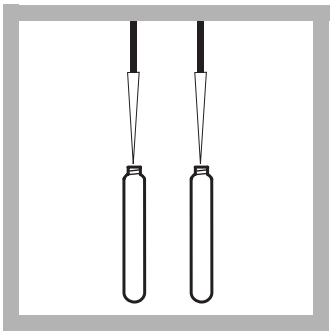
4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.



5. Label two Mid Range Acid Digestion vials *sample* and *reagent blank*.



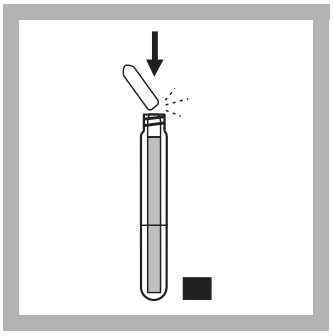
6. Use a funnel to add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).



7. Use a TenSette® Pipet to add 1.0 mL of organic-free water to the reagent blank vial and 1.0 mL of prepared sample to the sample vial. Swirl to mix.

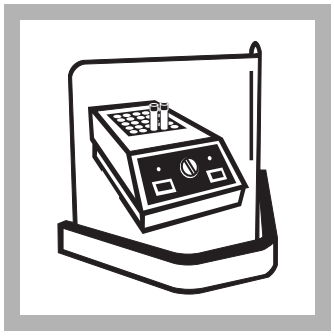
8. Rinse two blue Indicator Ampules with deionized water and wipe them with a soft, lint-free wipe.

Do not touch the ampules sides after wiping. Pick them up by the top.

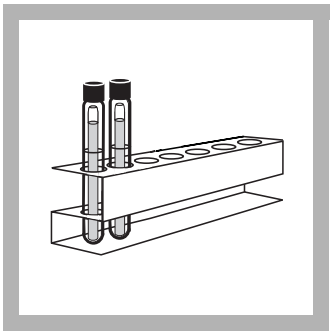


9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, snap the top off the ampule and allow it to drop into the Acid Digestion vial.

Do not invert or tilt the vial after inserting the ampule.



10. Cap the vial assemblies tightly and place them in the COD reactor for 2 hours at 103–105 °C.



11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for one hour for accurate results.

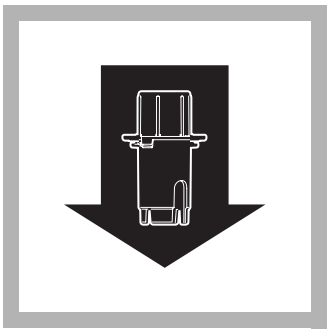
12. Touch

Hach Programs.

Select program

425 Organic Carbon MR.

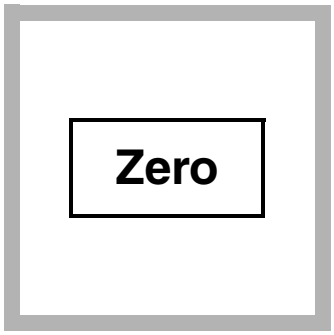
Touch **Start**.



13. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Wipe the reagent blank vial assembly and place it into the cell adapter.

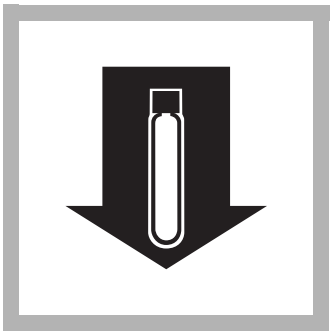


14. Touch **Zero**.

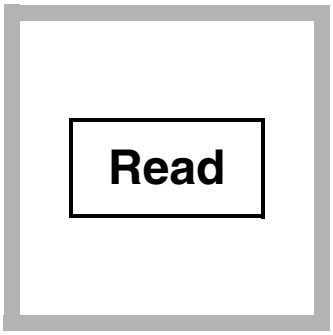
The display will show:

0 mg/L C

then **Underrange** until the sample is read.



15. Wipe the reagent vial assembly and place it into the cell adapter.



16. Touch **Read.**

Results will appear in mg/L C.

Note: *The display will show* **Underrange** *when the mg/L C value is below the lower limit of the method.*

Interferences

The following have been tested for interference and found not to interfere up to the indicated levels:

Substance	Maximum Level Tested
Aluminum	10 mg/L
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br
Bromine	25 mg/L Br_2
Calcium	2000 mg/L as $CaCO_3$
Chloride	1500 mg/L
Chlorine	10 mg/L Cl_2
Chlorine Dioxide	6 mg/L ClO_2
Copper	10 mg/L
Cyanide	10 mg/L CN
Iodide	50 mg/L
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Magnesium	2000 mg/L as $CaCO_3$
Manganese (VII)	1 mg/L
Monochloramine	14 mg/L NH_2Cl as Cl_2
Nitrite	500 mg/L NO_2^-
Ozone	2 mg/L O_3

(continued)

Substance	Maximum Level Tested
Phosphate	3390 mg/L PO_4^{3-}
Silica	100 mg/L SiO_2
Sulfate	5000 mg/L SO_4^{2-}
Sulfide	20 mg/L S^{2-}
Sulfite	50 mg/L SO_3^{2-}
Zinc	5 mg/L

If the sample contains greater than 1000 mg/L $CaCO_3$ alkalinity, lower the sample pH to less than 7 before testing by adding Sulfuric Acid Solution (Cat. No. 2449-32).

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 50 NTU have been tested without interference.

Sampling and Storage

Collect samples in clean glass bottles. Rinse the sample bottle several times with the sample to be collected. Fill the bottle completely full before capping. Test samples as soon as possible. Acid preservation is not recommended. Homogenize samples containing solids to assure representative samples.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Prepare a 300-mg/L C standard by transferring 15.00mL of a 1000-mg/L organic carbon to a 50 mL Class A volumetric flask. Dilute to volume using Organic-free Reagent Water. Stopper and mix thoroughly.
2. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of the 300-mg/L C standard to each of three Acid digestion vials.
3. Add the contents of one TOC Persulfate Powder Pillow to each vial.
4. Add 1.0 mL sample to each vial. Swirl to mix.
5. Proceed with the procedure starting at *step 8* on page *2*.
6. The mg/L C concentration should increase by 30 mg/L for each 0.1 mL increment.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

1. Prepare a 1000-mg/L organic carbon stock standard by dissolving 2.1254 g dry primary standard Potassium Acid Phthalate in Organic-Free Reagent Water and dilute to 1000 mL. This stock standard is stable for about 1 month at room temperature.

   Alternatively, open one ampule of TOC Standard Solution (Cat. No. 27915-05).
2. Prepare a 100-mg/L C standard by transferring 5.00 mL of the stock standard to a 50-mL Class A volumetric flask. Dilute to volume using Organic-free Reagent Water. Stopper and mix thoroughly. Prepare this standard fresh daily.

Method Performance

Precision

Program	mg/L C	95% Confidence Limits of Distribution
425	15	±6 mg/L C
425	50	±3 mg/L C
425	75	±2 mg/L C
425	115	±5 mg/L C
425	150	±6 mg/L C

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	1.4 mg/L C

See *Section 3.4.5 Sensitivity* on page *44* for more information.

To test for TOC levels below 15 mg/L C, use Method 10129. For more information on determining precision data and method detection limits, refer to *Section 3.4 Method Performance* on page *42*.

Summary of Method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial, organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The adsorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample. Test results are measured at 598 and 430 nm.

Organic Carbon, Total

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Reagent Set, Total Organic Carbon Direct Method Mid Range Test 'N Tube™		50 vials	28159-45
Includes:			
Acid Digestion Solution Vials, High Range TOC	1	50/pkg	*
Buffer Solution, Sulfate	0.4 mL	25 mL	452-33
Funnel, micro	1	each	25843-35
Indicator Ampules, High Range TOC	1	10/pkg	*
TOC Persulfate Powder Pillows	1	50/pkg	*
pH Paper	1	5/pkg	391-33
Water, Organic-free	3.0 mL	500 mL	26415-49

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
COD Reactor, 115/230 VAC (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 VAC (Europe)	1	each	45600-02
Cylinder, graduated, 10-mL	1	each	508-38
Flask, Erlenmeyer, 50-mL	1	each	505-41
Magnetic Stirrer	1	each	23436-00
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1–3	each	18641-00
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet	2	50/pkg	21997-96
Stir Bar, Magnetic	1	each	45315-00
Wipes, Disposable, Kimwipes®	1	280/pkg	20970-00

Required Standards

Potassium Acid Phthalate		500 g	315-34
TOC Standard Solution (KHP Standard, 1000-mg/L C)		5/pkg	27915-05

* Not sold separately.

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Organic Carbon, Total

Method 10129

Direct Method*

LR (0.3 to 20.0 mg/L C)

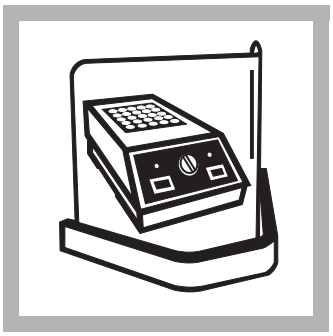
Scope and Application: For water, drinking water, and wastewater

* U.S. Patent 6,368,870

Tips and Techniques

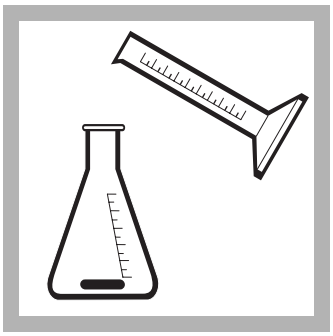
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- A reagent blank is required for each series of samples.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Method 10129



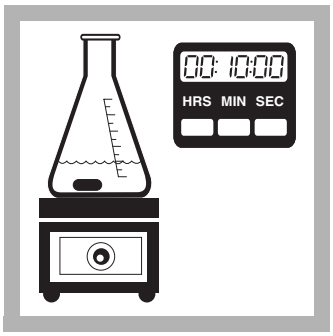
1. Turn on the COD reactor.

Heat to 103–105 °C.

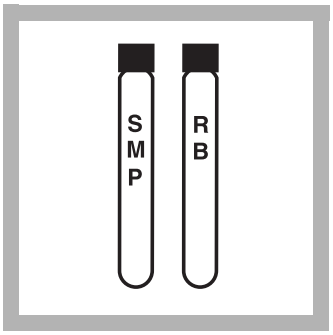


2. Use a graduated cylinder to add 10 mL of sample to a 50-mL Erlenmeyer flask that contains a stir bar.

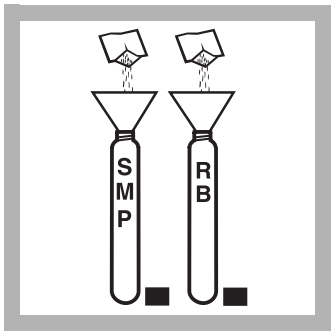
3. Add 0.4 mL of Buffer Solution, pH 2.0. Use pH paper to make sure the sample pH is 2.



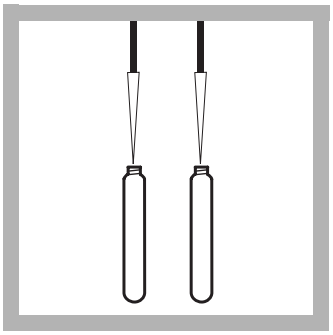
4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.



5. Label two Low Range Acid Digestion vials "sample" and "reagent blank".



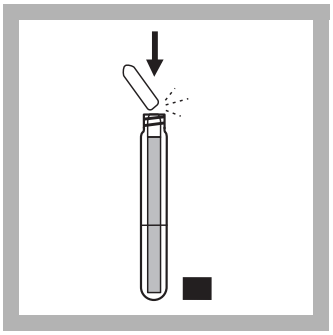
6. Use a funnel to add the contents of one TOC Persulfate Powder Pillow to each Acid Digestion vial (colorless liquid).



7. Use a TenSette® Pipet to add 3.0 mL of organic-free water to the reagent blank vial and 3.0 mL of prepared sample to the sample vial. Swirl to mix.

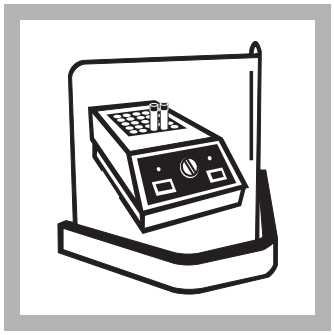
8. Rinse two blue Indicator Ampules with deionized water and wipe them with a soft, lint-free wipe.

Do not touch the ampules sides after wiping. Pick them up by the top.

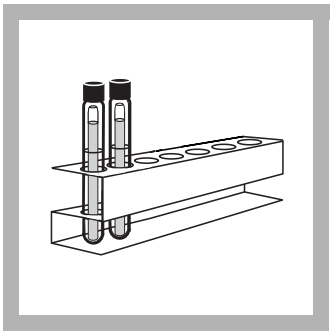


9. Lower one unopened ampule into each Acid Digestion vial. When the score mark on the ampule is level with the top of the Acid Digestion vial, snap the top off the ampule and allow it to drop into the Acid Digestion vial.

Do not invert or tilt the vial after inserting the ampule.



10. Cap the vial assemblies tightly and place them in the COD reactor for 2 hours at 103–105 °C.



11. Carefully remove the vial assemblies from the reactor. Place them in a test tube rack.

Allow the vials to cool for one hour for accurate results.

The liquid in the reagent blank vial should be dark blue.

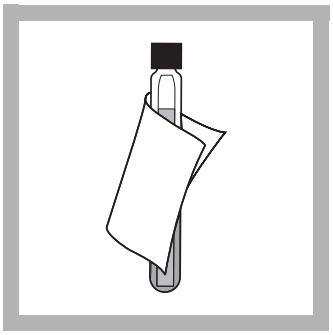
12. Touch

Hach Programs.

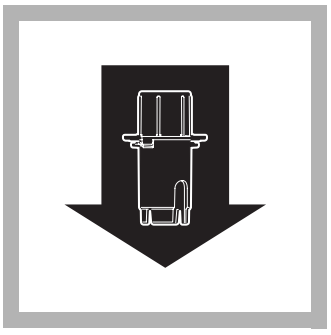
Select program

427 Organic Carbon LR.

Touch **Start**.



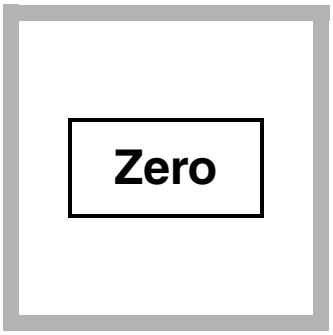
13. Wipe the reagent blank with a damp towel, followed by a dry one, to remove fingerprints or other marks.



14. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

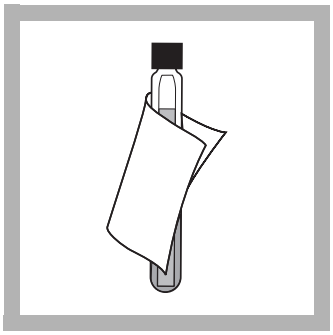
Place the reagent blank vial assembly in the cell adapter.



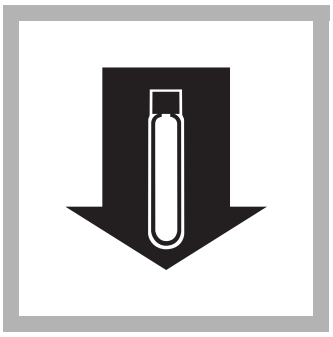
15. Touch **Zero**.

The display will show:

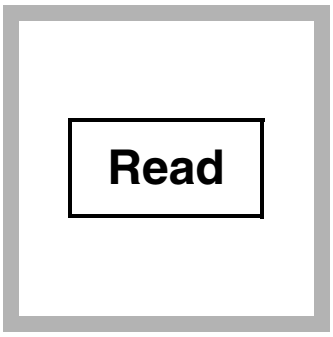
0.0 mg/L C.



16. Wipe the sample vial assembly with a damp towel, followed by a dry one, to remove fingerprints or other marks.



17. Place the sample vial assembly in the cell adapter.



18. Touch **Read**.

Results will appear in mg/L C.

Interferences

The following have been tested for interference and found not to interfere up to the indicated levels:

Substance	Maximum Level Tested
Aluminum	10 mg/L
Ammonia Nitrogen	1000 mg/L as N
ASTM Wastewater	No effect
Bromide	500 mg/L Br
Bromine	25 mg/L Br_2
Calcium	2000 mg/L as $CaCO_3$

(continued)

Substance	Maximum Level Tested
Chloride	500 mg/L
Chlorine	10 mg/L Cl_2
Chlorine Dioxide	6 mg/L ClO_2
Copper	10 mg/L
Cyanide	10 mg/L CN
Iodide	50 mg/L
Iron (II)	10 mg/L
Iron (III)	10 mg/L
Magnesium	2000 mg/L as $CaCO_3$
Manganese (VII)	1 mg/L
Monochloramine	14 mg/L NH_2Cl as Cl_2
Nitrite	500 mg/L NO_2^-
Ozone	2 mg/L O_3
Phosphate	3390 mg/L PO_4^{3-}
Silica	100 mg/L SiO_2
Sulfate	5000 mg/L SO_4^{2-}
Sulfide	20 mg/L S^{2-}
Sulfite	50 mg/L SO_3^{2-}
Zinc	5 mg/L

If the sample contains greater than 600 mg/L $CaCO_3$ alkalinity, lower the sample pH to less than 7 before testing by adding Sulfuric Acid Solution (Cat. No. 2449-32).

Most sample turbidity is either dissolved during the digestion stage or settled during the cooling period. Sample turbidities up to 50 NTU have been tested without interference.

Sampling and Storage

Collect samples in clean glass bottles. Rinse the sample bottle several times with the sample to be collected. Fill the bottle completely full before capping. Test samples as soon as possible. Acid preservation is not recommended. Homogenize samples containing solids to assure representative samples.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Prepare a 150-g/L C standard by transferring 15.00mL of 1000-mg/L C stock solution to a 100-mL Class A volumetric flask. Dilute to volume with organic-free water. Mix.
2. Use the TenSette® Pipet to add 0.1, 0.2, and 0.3 mL of the 150-mg/L C standard to each of three Acid Digestion Vials.
3. Add the contents of one TOC Persulfate Powder Pillow to each vial.
4. Add 3.0 mL of sample to each vial. Swirl to mix.

5. Continue the test starting at *step 8* on page 2 of this procedure.
6. The mg/L C concentration should increase by 5.0 mg/L for each 0.1 mL increment.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions Method

1. Prepare a 1000-mg/L organic carbon stock standard by dissolving 2.1254 g dry primary standard Potassium Acid Phthalate in Organic-free Reagent Water and dilute to 1000 mL. This stock standard is stable for about 1 month at room temperature.

   Alternatively, open one ampule of TOC Standard Solution (Cat. No. 27915-05).
2. Prepare a 10.0-mg/L C standard by transferring 10.00 mL of the stock standard to a 1000-mL Class A volumetric flask. Dilute to volume using Organic-Free Reagent Water. Stopper and mix thoroughly. Prepare this standard fresh daily.

Method Performance

Precision

Program	mg/L C	95% Confidence Limits of Distribution
427	1	±1.6 mg/L C
427	5	±1.1 mg/L C
427	10	±0.7 mg/L C
427	15	±0.6 mg/L C
427	20	±0.7 mg/L C

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Mid-range	0.010	0.3 mg/L C

See *Section 3.4.5 Sensitivity* on page *44 Sensitivity* for more information.

Summary of Method

The total organic carbon (TOC) is determined by first sparging the sample under slightly acidic conditions to remove the inorganic carbon. In the outside vial, organic carbon in the sample is digested by persulfate and acid to form carbon dioxide. During digestion, the carbon dioxide diffuses into a pH indicator reagent in the inner ampule. The adsorption of carbon dioxide into the indicator forms carbonic acid. Carbonic acid changes the pH of the indicator solution which, in turn, changes the color. The amount of color change is related to the original amount of carbon present in the sample. Test results are measured at 598 and 430 nm.

Organic Carbon, Total

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Total Organic Carbon Direct Method Low Range			
Test 'N Tube™ Reagent Set		50 vials	27603-45
Includes:			
Acid Digestion Solution Vials, Low Range TOC	1	50/pkg	*
Buffer Solution, Sulfate	0.4 mL	25 mL	452-33
Funnel, micro	1	each	25843-35
Indicator Ampules, Low Range TOC	1	10/pkg	*
TOC Persulfate Powder Pillows	1	50/pkg	*
pH Paper	1	5/pkg	391-33
Water, organic-free	3.0 mL	500 mL	26415-49

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
COD Reactor, 115/230 VAC (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 VAC (Europe)	1	each	45600-02
Cylinder, graduated, 10-mL	1	each	508-38
Flask, Erlenmeyer, 50-mL	1	each	505-41
Magnetic Stirrer	1	each	23436-00
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1–3	each	18641-00
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet	2	50/pkg	21997-96
Stir Bar, Magnetic	1	each	45315-00
Wipes, Disposable, Kimwipes	1	280/pkg	20970-00

Required Standards

Potassium Acid Phthalate		500 g	315-34
TOC Standard Solution (KHP Standard, 1000-mg/L C)		5/pkg	27915-05

* Not sold separately.

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Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Oxygen Demand, Chemical

★Method 8000 — Reactor Digestion Method*

(3 to 150, 20 to 1500, and 200 to 15,000 mg/L COD)

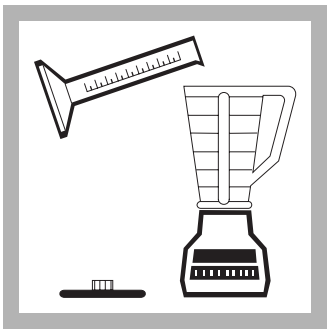
Scope and Application: For water, wastewater, and seawater; digestion is required; 3–150 mg/L and 20– 1500 mg/L COD ranges are USEPA approved for wastewater analyses**; 200–15,000 mg/L COD range is not USEPA approved.

* Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397
** *Federal Register*, April 21, 1980, 45(78), 26811-26812

Tips and Techniques

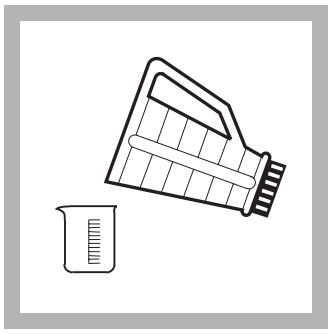
- Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings and refer to *Waste Management and Safety* on page *55* of this manual.
- Wear appropriate eye protection and clothing for adequate user protection. If contact occurs, flush the affected area with running water. Follow instructions carefully.
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- The reagent mixture is light-sensitive. Keep unused vials in the opaque shipping container. Refrigerate if possible.
- Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Wash spills with running water.
- Run one blank with each set of samples. Run all tests (the samples and the blank) with the same lot of vials. The lot number appears on the container label. See *Blanks for Colorimetric Determination* on page *4*.
- For greater accuracy, analyze a minimum of three replicates and average the results.

Digestion Procedure — Method 8000

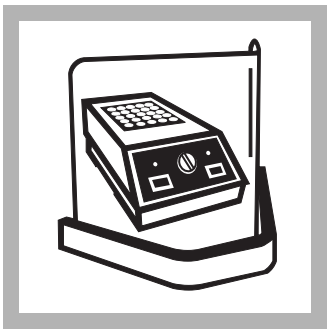


1. Homogenize 100 mL of sample for 30 seconds in a blender. (For samples containing large amounts of solids, increase the homogenization time.)

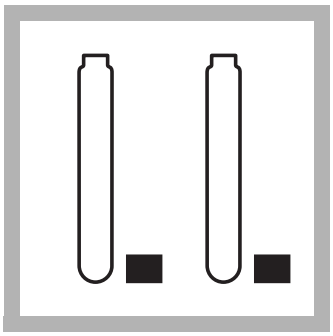
Note: *If the sample does not contain suspended solids, omit step 1 and step 2.*



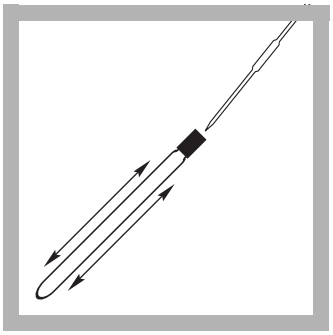
2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.



3. Turn on the COD Reactor. Preheat to 150 °C. Place the safety shield in front of the reactor.

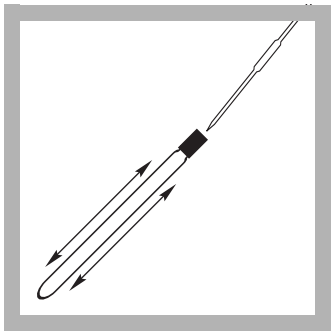


4. Remove the caps from two COD Digestion Reagent Vials. (Be sure to use vials for the appropriate range.)



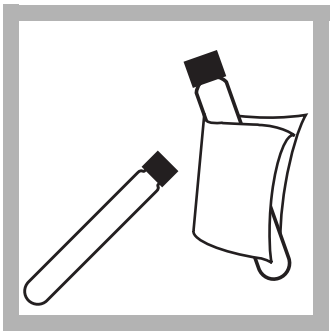
5. Hold one vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of sample to the vial. This is the prepared sample.

Note: *Use a TenSette pipet to add 0.20 mL for the 200–15,000 mg/L range.*

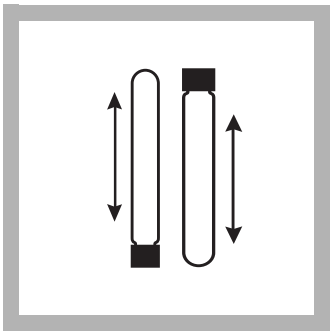


6. Hold a second vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of deionized water to the vial. This is the blank.

Note: *Use a TenSette pipet to add 0.20 mL for the 200–15,000 mg/L range.*

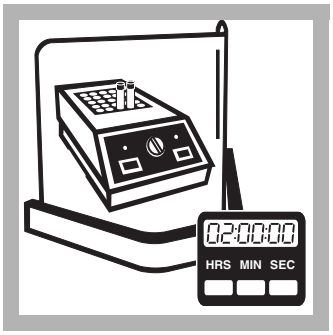


7. Cap the vials tightly. Rinse them with deionized water and wipe with a clean paper towel.

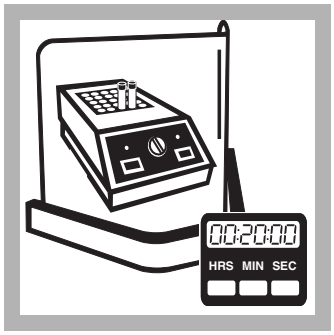


8. Hold the vials by the cap over a sink. Invert gently several times to mix. Place the vials in the preheated COD Reactor.

The sample vials will become very hot during mixing.

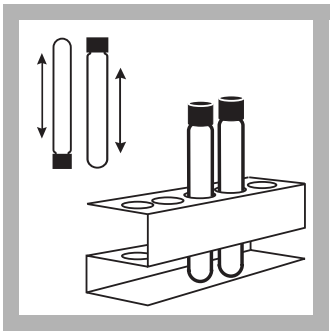


9. Heat the vials for two hours.



10. Turn the reactor off.

Wait about 20 minutes for the vials to cool to 120 °C or less.



11. Invert each vial several times while still warm. Place the vials into a rack and cool to room temperature.

12. Proceed to the *Colorimetric Determination Method 8000* on page 3.

Colorimetric Determination — Method 8000

1. Touch

Hach Programs.

Select program

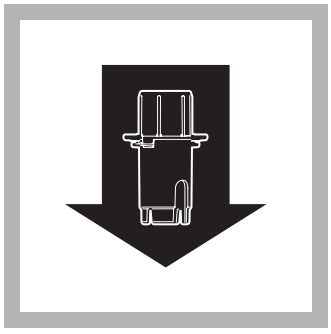
430 COD LR (Low Range)

or

435 COD HR (High Range/High Range Plus).

Touch **Start**.

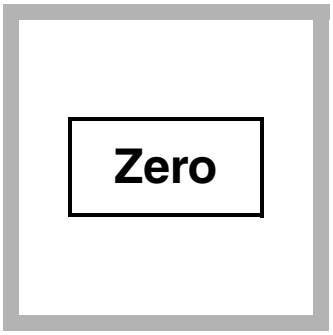
2. Clean the outside of the vials with a damp towel followed by a dry one to remove fingerprints or other marks.



3. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

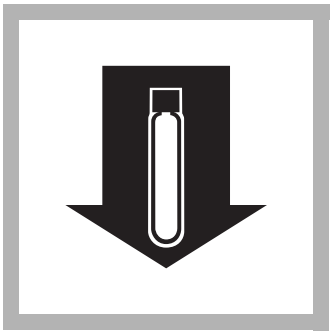
Place the blank into the adapter.



4. Touch **Zero**.

The display will show:

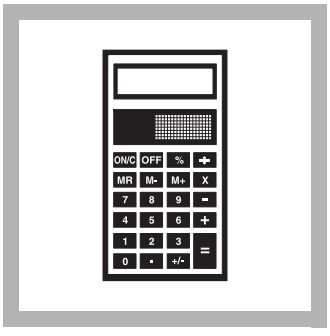
0 mg/L COD



5. When the timer beeps, place the sample vial into the adapter.

Touch **Read**.

Results will appear in mg/L COD.



6. If using High Range Plus COD Digestion Reagent Vials, multiply the result by 10.

Note: *For most accurate results with samples near 1,500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.*

Blanks for Colorimetric Determination

The blank may be used repeatedly for measurements using the same lot of vials. Store it in the dark. Monitor decomposition by measuring the absorbance at the appropriate wavelength (420 or 620 nm). Zero the instrument in the absorbance mode, using a vial containing 5 mL of deionized water and measure the absorbance of the blank. Record the value. Prepare a new blank when the absorbance has changed by about 0.01 absorbance units.

Interferences

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in column 1 in the table below. Samples with higher chloride concentrations should be diluted. Dilute the sample enough to reduce the chloride concentration to the level given in column 3.

If sample dilution will cause the COD concentration to be too low for accurate determination, add 0.50 g of mercuric sulfate ($HgSO_4$) (Cat. No. 1915-20) to each COD vial before the sample is added. The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in column 4.

Vial Type Used	Maximum Cl^- concentration in sample (mg/L)	Suggested Cl^- concentration of diluted samples (mg/L)	Maximum Cl^- concentration in sample when 0.50 $HgSO_4$ added
Low Range (3–150 mg/L)	2000	1000	8000
High Range (20–1500 mg/L)	2000	1000	4000
High Range Plus (200–15,000 mg/L)	20,000	10,000	40,000

Sampling and Storage

Collect samples in glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with sulfuric acid (Cat. No. 979-49) to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days. Correct results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29* for more information.

Accuracy Check

Standard Solution Method

1. Check the accuracy of the 3 to 150 mg/L range with a 100 mg/L standard. Prepare by dissolving 85 mg of dried (120 °C, overnight) potassium acid phthalate (KHP) in 1 liter of deionized water. Use 2 mL as the sample volume. The result should be 100 mg/L COD. Or dilute 10 mL of 1000-mg/L COD Standard Solution to 100 mL to produce a 100-mg/L standard.
   - To adjust the calibration curve using the reading obtained with the 100 mg/L COD standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
   - Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust.**
2. Check the accuracy of the 20 to 1,500 mg/L range by using either a 300 mg/L or 1000 mg/L COD Standard Solution. Use 2 mL of one of these solutions as the sample volume; the expected result will be 300 or 1000 mg/L COD respectively. Or, prepare a 500 mg/L standard by dissolving 425 mg of dried (120 °C, overnight) KHP. Dilute to 1 liter with deionized water.
   - To adjust the calibration curve using the reading obtained with the 300 mg/L or 1000 mg/L COD standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
   - Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust.**
3. Check the accuracy of the 200 to 15,000 mg/L range by using a 10,000 mg/L COD standard solution. Prepare the 10,000 mg/L solution by dissolving 8.500 g of dried (120 °C, overnight) KHP in 1 liter of deionized water. Use 0.2 mL of this solution as the sample volume; the expected result will be 10,000 mg/L COD.
   - To adjust the calibration curve using the reading obtained with the 10,000 mg/L COD standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
   - Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust.**

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 80 mg/L COD (Low Range), 800 mg/L COD (High Range), and 10,000mg/L COD (High Range Plus)

Program	Range (mg/L)	95% Confidence Limits of Distribution
430	3–150	77.6–82.4 mg/L COD
435	20–1500	778–822 mg/L COD
435	200–15000	9778–10,222 mg/L COD

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
430	Entire range	0.010	3 mg/L COD
435	Entire range	0.010	20 mg/L COD

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Alternate Reagents

Mercury-free COD2 Reagents can provide a mercury-free testing option for non-reporting purposes. For process control applications, COD2 Reagents will eliminate mercury waste and save on disposal costs. These reagents are fully compatible with test procedures and calibration curves programmed into the DR/2400 spectrophotometer. Determine chloride and ammonia for accurate results.

Note: *These reagents are not approved for USEPA reporting purposes. Request a copy of the COD Reagent Vial Information Brochure, Lit. No. 1356, for more information about specific applications.*

Summary of Method

The mg/L COD results are defined as the mg of O_2 consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}). When the 3–150 mg/L colorimetric or titrimetric method is used, the amount of Cr^{6+} remaining is determined. When the 20–1,500 mg/L or 200–15,000 mg/L colorimetric method is used, the amount of Cr^{3+} produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences. Test results for the 3 to 150 mg/L range are measured at 420 nm. Test results for the 20 to 1,500 and the 2000 to 15,000 mg/L COD range are measured at 620 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Select the appropriate COD Digestion Reagent Vial:			
Low Range, 0 to 150 mg/L COD	1 to 2 vials	25/pkg	21258-25
High Range, 0 to 1,500 mg/L COD	1 to 2 vials	25/pkg	21259-25
High Range Plus, 0 to 15,000 mg/L COD	1 to 2 vials	25/pkg	24159-25
Water, deionized	varies	4 L	272-56

Alternate Reagents*

Select the appropriate COD Digestion Reagent Vial:			
COD2, Low Range, 0 to 150 mg/L COD	1 to 2 vials	25/pkg	25650-25
COD2, High Range, 0 to 1,500 mg/L COD	1 to 2 vials	25/pkg	25651-25
COD2, High Range, 0 to 1,500 mg/L COD	1 to 2 vials	150/pkg	25651-15
COD2, High Range Plus, 0 to 15,000 mg/L COD	1 to 2 vials	25/pkg	28343-25

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Blender, 2-speed, 120 VAC	1	each	26161-00
Blender, 2-speed, 240 VAC	1	each	26161-02
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet, Volumetric, Class A, 2.00 mL	1	each	14515-36
Pipet Filler, safety bulb	1	each	14651-00
Reactor, COD, 120/240 Vac, North American fuses/plug	1	each	45600-00
Reactor, COD, 120/240 Vac, European fuses and plug	1	each	45600-02
Stirrer, Electromagnetic, 120 VAC, with electrode stand	1	each	45300-01
Stirrer, Electromagnetic, 230 VAC, with electrode stand	1	each	45300-02
Test Tube Rack	1 to 2 racks	each	18641-00

Required Standards

COD Digestion Reagent Vials, 0 to 150 mg/L COD	150/pkg	21258-15
COD Digestion Reagent Vials, 0 to 1,500 mg/L COD	150/pkg	21259-15
COD Standard Solution, 300-mg/L	200 mL	12186-29
COD Standard Solution, 1000-mg/L	200 mL	22539-29
Potassium Acid Phthalate, ACS	500 g	315-34

*These reagents are not approved for USEPA reporting purposes. Request a copy of the COD Reagent Vial Information Brochure, Lit. No. 1356, for more information about specific applications.

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Method 10067

Manganese III Reactor Digestion Method (with optional chloride removal)*

(30 to 1000 mg/L COD Mn)

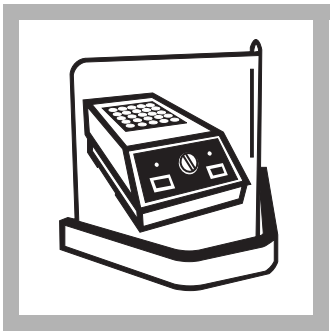
Scope and Application: For water and wastewater

* U.S. Patent 5,556,787

Tips and Techniques for Preparing the Acidified Sample

- Use this method if the sample contains chloride. To determine if the sample contains chloride, use Quantab® Titrator Strips for low range chloride (Cat. No. 27449-40).
- If the sample COD is expected to exceed 1000 mg/L, dilute the sample as described in *Table 1* on page *2*.
- Blending the sample promotes even distribution of solids and improves accuracy and reliability.
- Run one blank with each lot of reagent. Run all samples and blanks with the same lot of vials. The lot number appears on the container label.
- The stability of the reagent blank allows for reuse. Verify the reagent blank quality by measuring the absorbance of the blank vs. a clean COD vial filled with deionized water. The absorbance range should be about 1.41–1.47.
- If the sample boils during the digestion, the vial is not properly sealed. Test results will be invalid.
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- Spilled reagent will affect test accuracy and is hazardous. Do not run tests with spilled vials.

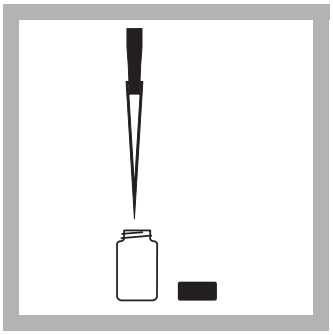
Preparing the Acidified Sample



1. Turn on the COD Reactor and heat to 150 °C. Place the safety shield in front of the reactor.

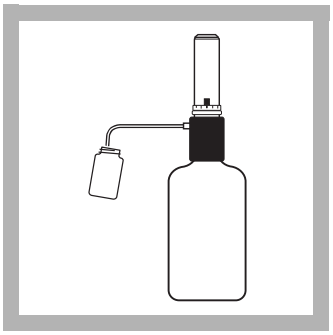
2. Homogenize 100 mL of sample for 30 seconds in a blender.

If suspended solids are present, continue to mix the sample while pipetting.

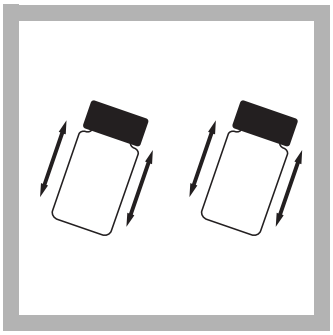


3. Pipet 9.0 mL of homogenized sample into an empty glass mixing cell (the prepared sample) and 9.0 mL of deionized water into another empty glass mixing cell (the blank).

Note: *Mixing concentrated sulfuric acid and water is not additive. Adding 1.0 mL of concentrated sulfuric acid to 9.0 mL of sample does not result in a final volume of 10.0 mL. This factor is built into the calibration curve.*



4. Using an automatic dispenser (Cat. No. 25631-37) or TenSette® Pipet, add 1.0 mL of concentrated sulfuric acid to both the sample cell and the blank.



5. Cap the cells tightly and invert several times.

The solution will become hot. Cool to room temperature before proceeding.

Acidified samples are stable for several months when refrigerated at 4 °C.

6. Proceed to *Tips and Techniques for Using the Vacuum Pretreatment Device* on page *3*.

Calculating the Multiplication Factor

All dilutions require that the ratio of sample to sulfuric acid remain at 9:1. For other dilutions that are not listed in *Table 1*, add the sample volume and the deionized water and divide by the sample volume to obtain the multiplication factor.

Table 1

Sample (mL)	Deionized Water (mL)	Range (mg/L COD)	Multiplication Factor
6.0	3.0	30–1500	1.5
3.0	6.0	60–3000	3
1.0	8.0	180–9000	9
0.5	8.5	360–18,000	18

For best results, use 0.5 mL or more of sample for diluting. If sample values exceed 18,000 mg/L COD, use a separate sample dilution before performing the sample chloride removal procedure.

Example: Dilute the sample to a range of 90–4500 mg/L COD.

Sample Volume (2.0 mL) + Deionized water (7.0 mL) = Total Volume (9.0 mL)

$$\text{Multiplication Factor} = \frac{\text{Total Volume}}{\text{Sample Volume}} = \frac{9.0\ \text{mL}}{2.0\ \text{mL}} = 4.5$$

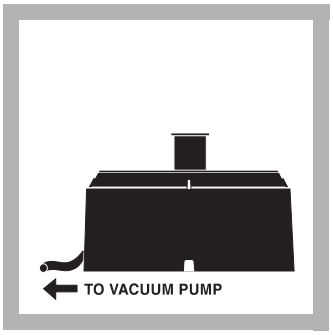
Standard test range is 50 to 1000 mg/L COD.

Example test range = 4.5(50) to 4.5(1000) = 225 to 4500 mg/L COD

Tips and Techniques for Using the Vacuum Pretreatment Device

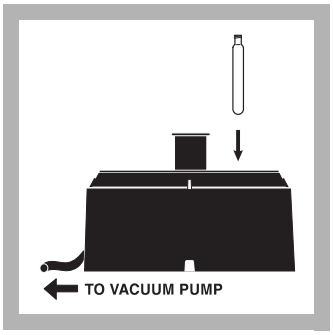
- If the sample does not flow through the Chloride Removal Cartridge (CRC), increase the vacuum until flow starts, then reduce the vacuum down to 20 inches of water. Proceed as usual.
- The maximum range of the VPD gauge is 40 inches of water; it will not indicate the full vacuum level obtained. Full vacuum is 20–25 inches of mercury; this can be measured at the vacuum pump with a gauge calibrated for inches of mercury.
- Dispose of the used Chloride Removal Cartridge. Do not reuse it.

Using the Vacuum Pretreatment Device

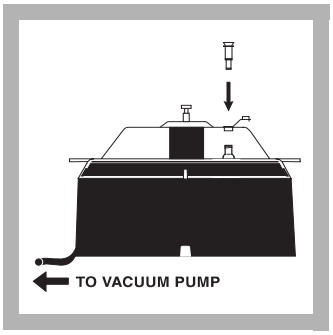


1. Attach the Vacuum Pretreatment Device* (VPD) to a vacuum pump (not an aspirator-type vacuum) that can create a vacuum of 20–25 inches of mercury.

*Patent Pending

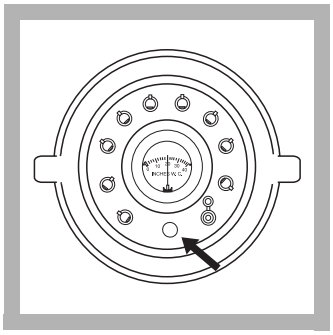


2. Label each Mn III COD vial and remove the cap. Place the vials in one of the numbered holes in the VPD base.

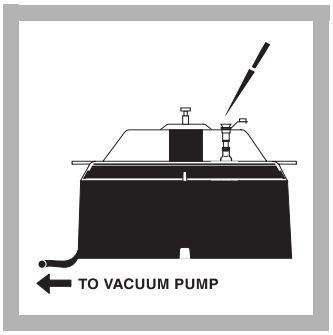


3. Place the VPD top on the base. Insert a fresh Chloride Removal Cartridge** (CRC) directly above each Mn III COD Reagent Vial. Plug any open holes in the VPD top using the stoppers provided.

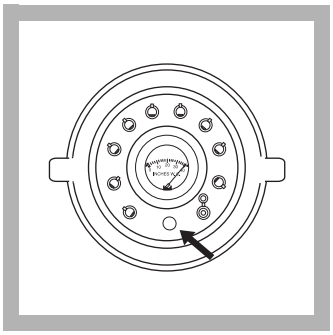
**U.S. Patents 5,667,754; 5,683,914



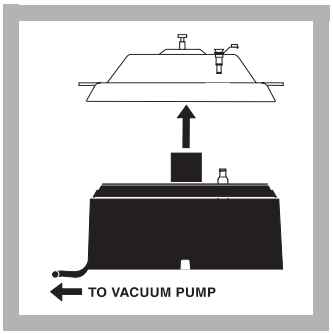
4. Turn on the vacuum pump and adjust the vacuum regulator valve on top of the VPD until the internal gauge reads 20 inches of water.



5. Pipet 0.60 mL of acidified sample (see *Preparing the Acidified Sample* on page *1*) into the CRC. Pipet 0.6 mL of acidified blank into another CRC. It should take 30–45 seconds to draw the liquid through the CRC into each vial.



6. Close the vacuum regulator valve completely to achieve full vacuum. After one minute of full vacuum, slide the VPD back and forth several times to dislodge any drops clinging to the cartridge.



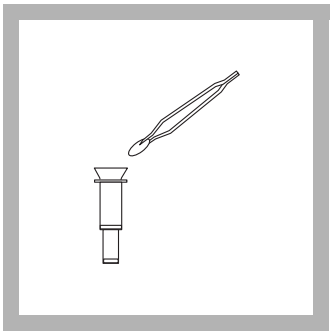
7. Open the VPD regulator valve to release the vacuum. Turn the pump off. Remove the VPD top and set it beside the base.

8. Proceed to *Tips and Techniques for Sample Preparation and Measurement*.

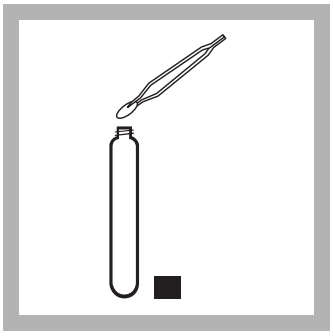
Tips and Techniques for Sample Preparation and Measurement

- To avoid cross-contamination between samples, clean forcep tips between samples by wiping with a clean towel or rinsing with deionized water.
- If the sample does not contain suspended solids, it is not necessary to transfer the filter to the digestion vial.
- If the sample boils during the digestion, the vial is not properly sealed. Test results will be invalid.
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- To oxidize resistant organics, you can digest samples for up to four hours. Digest the blank for the same time period as the samples.

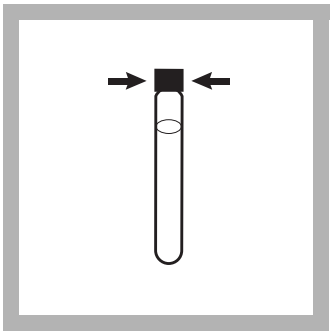
Sample Preparation and Measurement



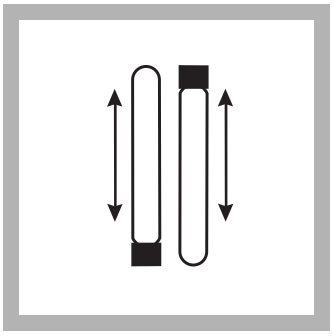
1. Use forceps to remove the filter from the top of each CRC.



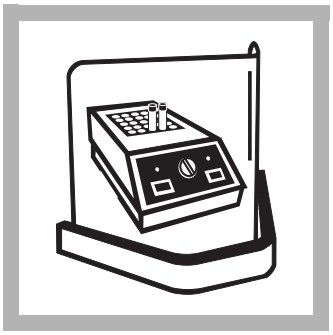
2. Place each filter in the corresponding Mn III COD Vial. (Use numbers on the VPD as a guide.)



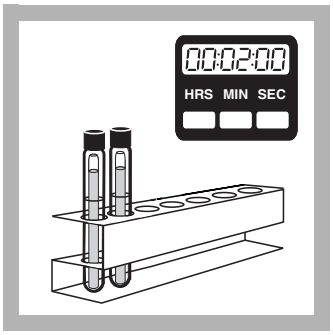
3. Remove the Mn III COD vial from the vacuum chamber and replace the original cap. Screw the cap on tightly.



4. Invert the vials several times to mix.

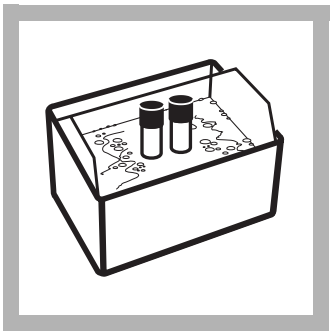


5. Place the vials in the COD Reactor at 150 °C. Digest for one hour.

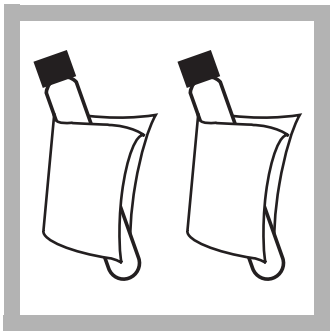


6. Place the vials in a cooling rack for two minutes.

If the solution develops a colorless upper layer and a purple lower layer, invert the vial several times to mix and proceed to the next step.



7. Cool the vials to room temperature in a cool water bath or with running tap water for several minutes.



8. Remove the vials from the water and wipe with a clean, dry paper towel.

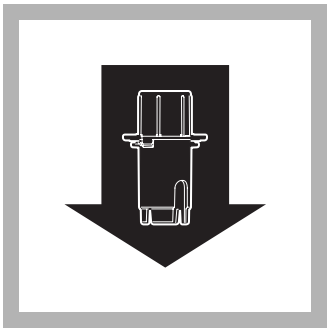
9. Touch

Hach Programs.

Select program

432 COD Mn III.

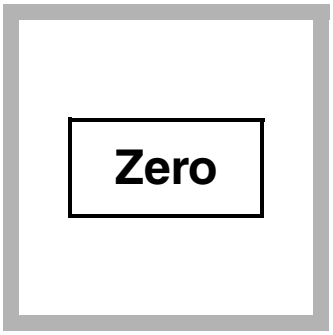
Touch **Start**.



10. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

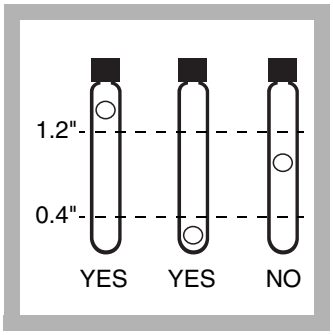
Place the blank into the adapter.



11. Touch **Zero**.

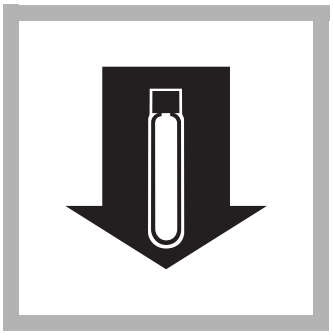
The display will show:

0 mg/L COD Mn

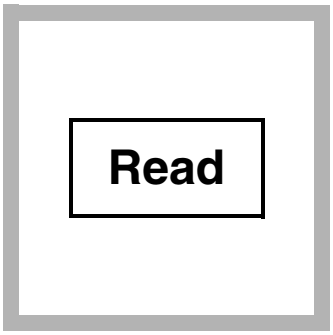


12. Make sure the filter disc is not suspended in the middle of the vial; it can interfere with the instrument reading.

Note: *The disc must be more than 30 mm (1.2"), or less than 10 mm (0.4"), from the bottom of the vial. Move it by gently swirling, or by lightly tapping the vial on the table top.*



13. Wipe the prepared sample and place it into the adapter.



14. Touch **Read.**

Results will appear in mg/L COD Mn.

Interferences

Inorganic materials may also be oxidized by trivalent manganese and constitute a positive interference when present in significant amounts. Chloride is the most common interference and is removed by sample pretreatment with the Chloride Removal Cartridge. If chloride is known to be absent or present in insignificant levels, the pretreatment can be omitted. A simple way to determine if chloride will affect test results is to run routine samples with and without the chloride removal, then compare results. Other inorganic interferences (i.e., nitrite, ferrous iron, sulfide) are not usually present in significant amounts. If necessary, these interferences can be corrected after determining their concentrations with separate methods and adjusting the final COD test results accordingly.

Ammonia nitrogen is known to interfere in the presence of chloride; it does not interfere if chloride is absent.

Sample Collection, Preservation, and Storage

Collect samples in clean glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with concentrated sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C may be stored up to 28 days. Correct results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Solution Method

1. Purchase an 800-mg/L COD Standard Solution directly from Hach or prepare an 800-mg/L COD standard solution by adding 0.6808 g of dried (103 °C, overnight) potassium acid phthalate (KHP) to 1 liter of deionized water. Use 0.50 mL of this solution (0.60 mL for the chloride removal procedure) as the sample volume.
2. To adjust the calibration curve using the reading obtained with the 800-mg/L COD standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.

3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Note: *Data is for Manganese III COD without the chloride removal procedure.*

Precision
Standard: 500 mg/L COD

Program	95% Confidence Limits of Distribution
432	479–521 mg/L COD

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	8 mg/L COD

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Chemical Oxygen Demand (COD) is defined as "... a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant" (APHA Standard Methods, 19th ed., 1995). Trivalent manganese is a strong, non-carcinogenic chemical oxidant that changes quantitatively from purple to colorless when it reacts with organic matter. It typically oxidizes about 80% of the organic compounds. Studies have shown that the reactions are highly reproducible and test results correlate closely to Biochemical Oxygen Demand (BOD) values and hexavalent chromium COD tests. None of the oxygen demand tests provide 100% oxidation of all organic compounds.

A calibration is provided which is based on the oxidation of Potassium Acid Phthalate (KHP). A different response may be seen in analyzing various wastewaters. The KHP calibration is adequate for most applications. The highest degree of accuracy is obtained when test results are correlated to a standard reference method such as BOD or one of the chromium COD methods. Special waste streams or classes will require a separate calibration to obtain a direct mg/L COD reading or to generate a correction factor for the precalibrated KHP response. The sample digestion time can be extended up to four hours for samples that are difficult to oxidize. Test results are measured at 510 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Chloride Removal Cartridges (CRC)	1	25/pkg	26618-25
Manganese III COD Reagent Vials, 20–1000 mg/L COD	1	25/pkg	26234-25
Sulfuric Acid, concentrated, ACS	1 mL	2.5 L	979-09
Water, deionized	varies	4 L	272-56

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Blender, 120 VAC	1	each	26747-00
Blender Container, 50–250 mL	1	2/pkg	26748-00
Cap, with inert Teflon liner, for mixing bottle	varies	12/pkg	24018-12
COD Reactor, 115–230 VAC, 50-60 Hz	1	each	45600-00
or COD Reactor, 230 VAC, 50 Hz	1	each	45600-02
Forceps, extra fine point	1	each	26696-00
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	2	50/pkg	21997-96
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette®	2	50/pkg	21856-96
Safety Shield	1	each	50030-00
Test Tube Rack, COD	1	each	18641-00
Vacuum Pretreatment Device (VPD)	1	each	49000-00
Vacuum Pump, 1.2 CFM @ 60Hz. 27.2 Hg Max, 1/8 HP, UL listed	1	each	28248-00
Vial, glass, for sample + acid	2	each	24277-00

Required Standards

COD Standard Solution, 800-mg/L COD	200 mL	26726-29
Potassium Acid Phthalate, ACS	500 g	315-34

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Oxygen Demand, Chemical

Method 10067

Manganese III Reactor Digestion Method (without chloride removal)*

(30 to 1000 mg/L COD Mn)

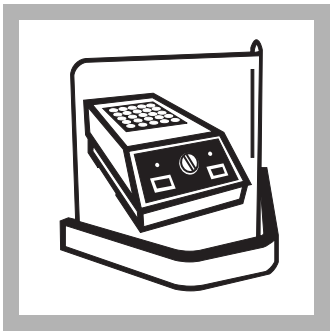
Scope and Application: For water and wastewater

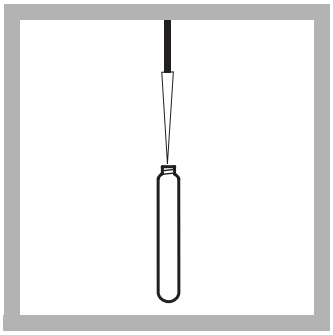
* U.S. Patent 5,556,787

Tips and Techniques

- For information about sampling, storage, accuracy checks, interferences, method summary, reagents and apparatus, see *Manganese III Reactor Digestion Method (with optional chloride removal)*.
- If the sample contains chloride, use the chloride removal method. To determine if the sample contains chloride, use Quantab® Titrator Strips for low range chloride (Cat. No. 27449-40).
- If the sample COD value is not between 30 and 1000 mg/L, dilute the sample with deionized water to obtain this range. Multiply the final result by the dilution factor.
- Blending the sample promotes even distribution of solids and improves accuracy and reliability.
- Stability of the reagent blank allows for reuse. Verify the reagent blank quality by measuring the absorbance of the blank vs. a clean COD vial filled with deionized water. The absorbance range should be about 1.4–1.5.
- If the sample boils during the digestion, the vial is not properly sealed. Test results will be invalid.
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- Spilled reagent will affect test accuracy and is hazardous. Do not run tests with spilled vials.

Without Chloride Removal — Method 10067





1. Touch

Hach Programs.

Select program

432 COD Mn III.

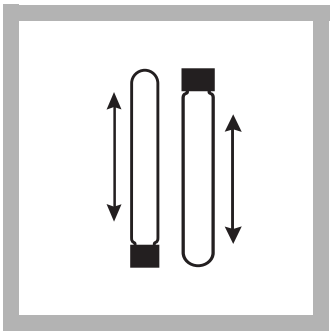
Touch **Start**.

2. Turn on the COD Reactor and heat to 150 °C. Place the safety shield in front of the reactor.

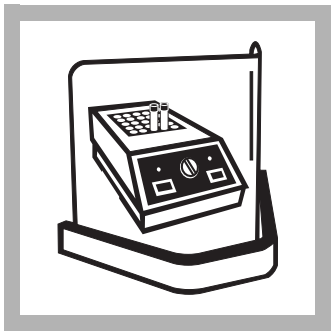
3. Homogenize 100 mL of sample for 30 seconds in a blender.

If suspended solids are present, continue to mix the sample while pipetting.

4. Pipet 0.5 mL of homogenized sample into one Mn III COD vial (the prepared sample) and 0.5 mL of deionized water into another Mn III COD vial (the blank).

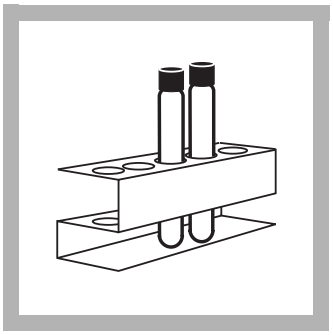


5. Cap and invert several times to mix.



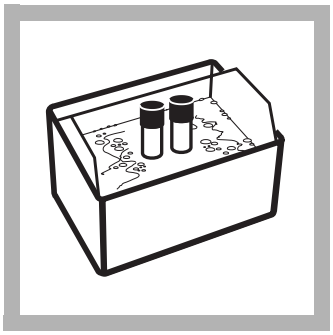
6. Place the vials in the preheated COD Reactor. Digest for 1 hour.

You may digest more resistant organics and the blank for up to four hours.

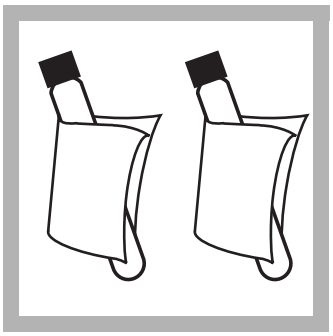


7. Remove the vials and place them in a cooling rack for two minutes.

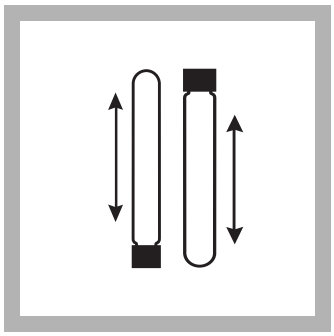
Note: *If a vial develops a colorless upper layer and a purple lower layer, invert the vial several times to mix and proceed.*



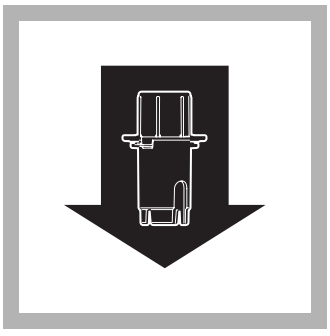
8. Cool the vials to room temperature in a cool water bath or with running tap water. This takes several minutes.



9. Remove the vials from the water and wipe with a clean, dry paper towel.

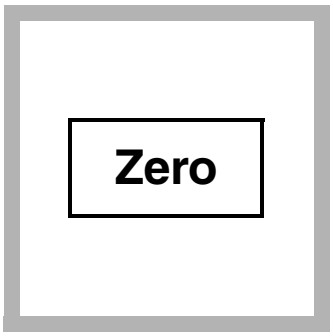


10. Invert the vials several times to mix.



11. Install the 16-mm adapter.

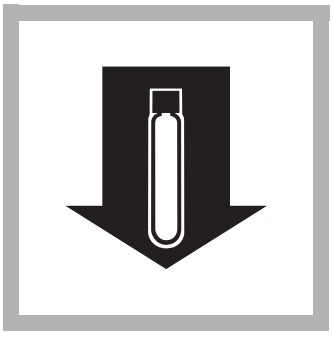
Place the blank into the adapter.



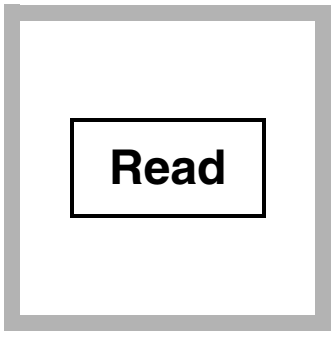
12. Touch **Zero**.

The display will show:

0 mg/L COD Mn



13. Place the sample into the cell holder.



14. Touch **Read.**

Results will appear in mg/L COD Mn.

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Oxygen, Dissolved

Method 8166 **HRDO Method**

AccuVac® Ampuls **HR (0.3 to 15.0 mg/L O_2)**

Scope and Application: For water and wastewater

Tips and Techniques

• Analyze samples on-site. Do not store for later analysis.

AccuVac Ampul Method 8166

1. Touch

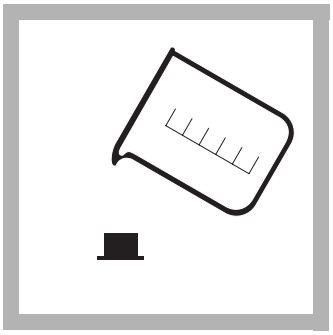
Hach Programs.

Select program

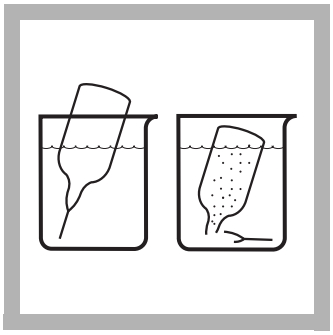
445 Oxygen Di. HR AV.

Touch **Start**.

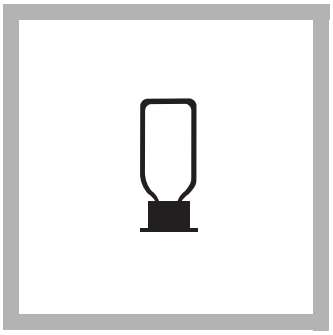
2. Fill a round sample cell (the blank) with at least 10-mL of sample.



3. Fill a blue ampule cap with sample.

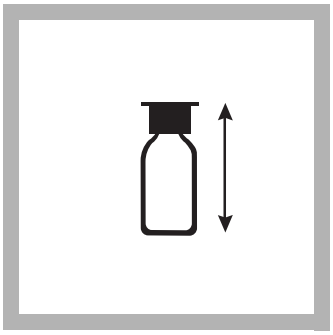


4. Fill a High Range Dissolved Oxygen AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



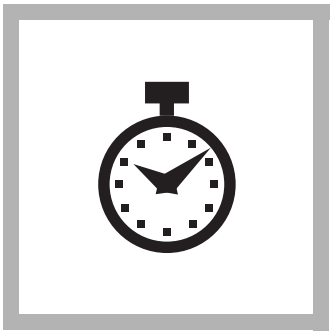
5. Hold the ampule with the tip pointing down and immediately place the ampule into the ampule cap.

The cap prevents contamination from atmospheric oxygen.



6. Shake the ampule for 30 seconds.

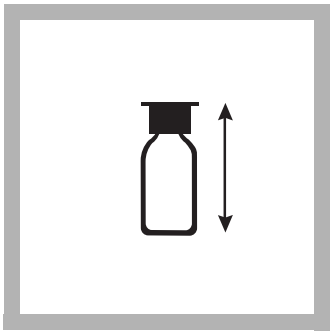
A small amount of undissolved reagent will not affect results.



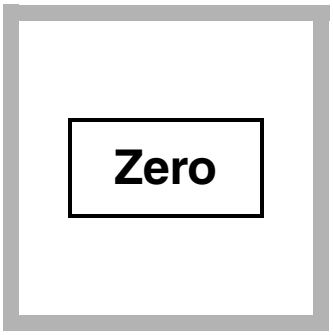
7. Touch the timer icon.

Touch **OK**.

A two-minute reaction period will begin. This enables the oxygen that was degassed during aspiration to redissolve and react.



8. When the timer beeps, shake the ampule for 30 seconds.



9. Place the blank in the cell holder.

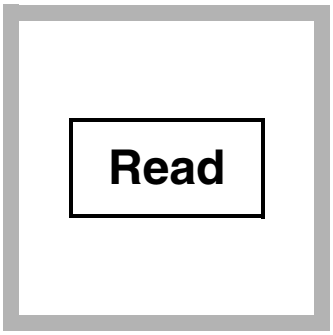
Touch **Zero**.

The display will show:

0.0 mg/L O_2

10. Place the ampule into the cell holder.

11. Wait approximately 30 seconds for the air bubbles to disperse from the light path.



12. Touch **Read.**

Results will appear in mg/L O_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Cr^{3+}	Greater than 10 mg/L
Cu^{2+}	Greater than 10 mg/L
Fe^{2+}	Greater than 10 mg/L
Mg^{2+}	Magnesium is commonly present in seawater and causes a negative interference. If the sample contains more than 50% seawater, the oxygen concentration obtained by this method will be 25% less than the true oxygen concentration. If the sample contains less than 50% seawater, the interference will be less than 5%.
Mn^{2+}	Greater than 10 mg/L
Ni^{2+}	Greater than 10 mg/L
NO_2^-	Greater than 10 mg/L

Sample Collection, Preservation, and Storage

The main consideration in sampling with the High Range Dissolved Oxygen Ampul is to prevent the sample from becoming contaminated with atmospheric oxygen between breaking open the ampule and reading the absorbance. This is accomplished by capping the ampule with an ampule cap. If the ampule is securely capped, the ampule should be safe from contamination for several hours. The absorbance will decrease by approximately 3% during the first hour and will not change significantly afterwards.

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested may change with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time, and other factors. A single dissolved oxygen test rarely reflects the accurate overall condition of a body of water. Several samples taken at different times, locations, and depths are recommended for most reliable results. Samples must be tested immediately upon collection, although only a small error results if the absorbance reading is taken several hours later.

Accuracy Check

The results of this procedure may be compared with the results of a titrimetric procedure (request Lit. Code 8042) or *sens**ion***™*6* Dissolved Oxygen Meter (Cat. No. 51850-01).

Method Performance

Precision

Standard: 7.9 mg/L O_2

Program	95% Confidence Limits of Distribution
445	7.5–8.3 mg/L O_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.09 mg/L O_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The High Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum sealed in a 14-mL ampule. When the AccuVac Ampul is opened in a sample containing dissolved oxygen, it forms a yellow color which turns purple. The purple color development is proportional to the concentration of dissolved oxygen. Test results are measured at 535 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
High Range Dissolved Oxygen AccuVac® Ampuls, 0–10 mg/L with 2 reusable ampule caps	1 ampul	25/pkg	25150-25

Required Apparatus

Polypropylene Beaker, 50-mL, Low Form, with pour spout		each	1080-41
Sample Cells, 10-mL, w/cap	1	6/pkg	24276-06

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3/04 1ed

Oxygen, Dissolved

Method 8316

Indigo Carmine Method

AccuVac® Ampuls

LR (6 to 800 µg/L O_2)

Scope and Application: For boiler feedwater

Tips and Techniques

• The ampules will contain a small piece of wire to maintain reagent quality. The solution will be yellow.

AccuVac Ampul — Method 8316

1. Touch

Hach Programs.

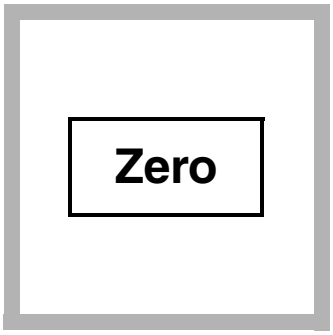
Select program

446 Oxygen Di. LR AV.

Touch **Start**.

2. Fill a round sample cell (the blank) with at least 10-mL of sample.

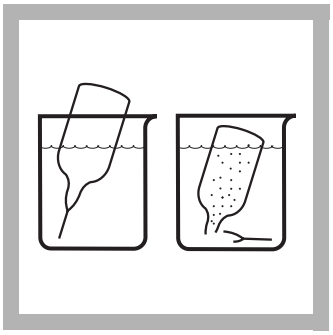
3. Place the blank into the cell holder.



4. Touch **Zero**.

The display will show:

0 µg/L O_2



5. Fill a Low Range Dissolved Oxygen Ampul with sample. Keep the tip immersed while the ampule fills completely.

6. Immediately place the ampule into the cell holder.

Touch **Read.**

Results will appear in µg/L O_2.

7. Use the initial reading. The reading is stable for 30 seconds. After 30 seconds the ampule solution will absorb oxygen from the air.

Interferences

Interfering Substance	Interference Levels and Treatments
Hydrazine	100,000 fold excess will begin to reduce the oxidized form of the indicator solution.
Sodium hydrosulfite	Reduces the oxidized form of the indicator solution and will cause a significant interference.

Excess amounts of thioglycolate, ascorbate, ascorbate + sulfite, ascorbate + cupric sulfate, nitrite, sulfite, thiosulfate, and hydroquinone will not reduce the oxidized form of the indicator and do not cause significant interference.

Sample Collection, Preservation, and Storage

The main consideration in this procedure is to prevent contaminating the sample with atmospheric oxygen. Sampling from a stream of water that is hard plumbed to the sample source is ideal. Use a funnel to maintain a continual flow of sample and yet collect enough sample to immerse the ampule. It is important not to introduce air in place of the sample. Rubber tubing, if used, will introduce unacceptable amounts of oxygen into the sample unless the length of tubing is minimized and the flow rate is maximized. Flush the sampling system with sample for at least 5 minutes.

Accuracy Check

The reagent blank for this test can be checked by following these steps:

1. Fill a 50-mL beaker with sample and add approximately 50 mg sodium hydrosulfite.
2. Immerse the tip of a Low Range Dissolved Oxygen AccuVac Ampul in the sample into the tip. Aspirate the sample into the ampule.
3. Determine the dissolved oxygen concentration according to the preceding procedure. The result should be 0 ±6 µg/L.

Summary of Method

The Low Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum sealed in a 14-mL ampule. When the AccuVac Ampul is broken open in a sample containing dissolved oxygen, the yellow solution will turn blue. The blue color development is proportional to the concentration of dissolved oxygen. Test results are measured at 610 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Low Range Dissolved Oxygen AccuVac® Ampuls	1	25/pkg	25010-25

Required Apparatus

Sample Cells, 10-mL, w/cap	1	6/pkg	24276-06

Required Standards

Sodium Hydrosulfite, technical-grade		500 g	294-34

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Oxygen, Dissolved

Method 8333

Ultra High Range Method

AccuVac® Ampul

UHR (1.0 to 40.0 mg/L O_2)

Scope and Application: For aquaculture

Tips and Techniques

- Analyze samples on-site. Do not store for later analysis.

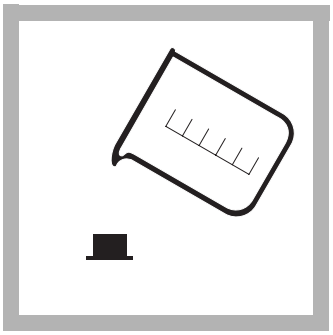
AccuVac Ampul — Method 8333

1. Touch **Hach Programs**.

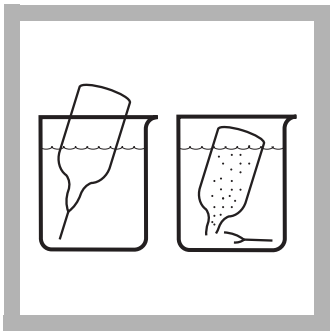
Select program **448 Oxygen Di. UHR AV**.

Touch **Start**.

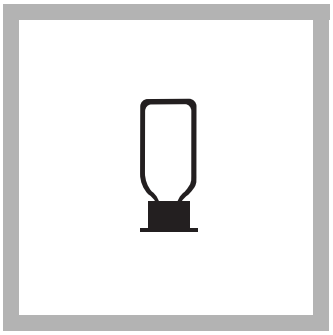
2. Fill a round sample cell with at least 10 mL of sample (the blank).



3. Fill a blue ampule cap with sample.

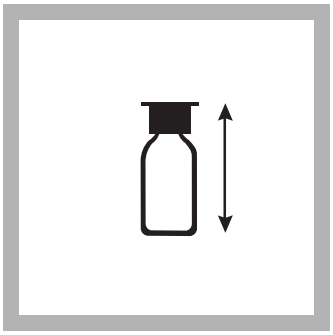


4. Fill a High Range Dissolved Oxygen AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



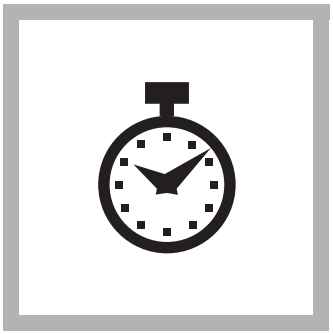
5. Hold the ampule with the tip pointing down and immediately place the ampule into the ampule cap.

The cap prevents contamination from atmospheric oxygen.



6. Shake the ampule for 30 seconds.

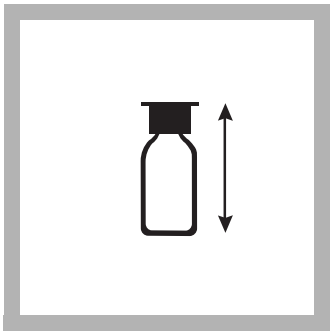
A small amount of undissolved reagent will not affect results.



7. Touch the timer icon.

Touch **OK**.

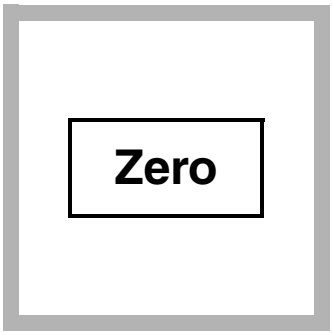
A two-minute reaction period will begin. This enables the oxygen that was degassed during aspiration to redissolve and react.



8. When the timer beeps, shake the ampule for 30 seconds.

Note: *Allow any bubbles to dissipate before proceeding.*

9. Place the blank into the cell holder.

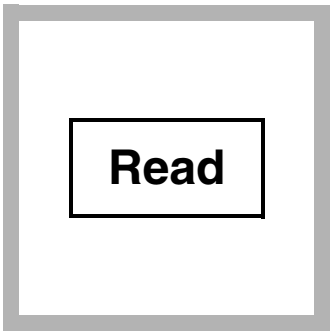


10. Touch **Zero**.

The display will show:

0.0 mg/L O_2

11. Place the AccuVac Ampul into the cell holder.



12. Touch **Read.**

Results will appear in mg/L O_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Cr^{3+}	Greater than 10 mg/L
Cu^{2+}	Greater than 10 mg/L
Fe^{2+}	Greater than 10 mg/L
Mg^{2+}	Magnesium is commonly present in seawater and interferes. If the sample contains more than 50% seawater, the oxygen concentration observed will be 25% lower than the true oxygen concentration. If the sample contains less than 50% seawater, the interference will be less than 5%.
Mn^{2+}	Greater than 10 mg/L
Ni^{2+}	Greater than 10 mg/L
NO_2^-	Greater than 10 mg/L

Sample Collection, Preservation, and Storage

The main consideration in sampling with the High Range Dissolved Oxygen AccuVac Ampul is to prevent the sample from becoming contaminated with atmospheric oxygen between breaking open the ampule and reading the absorbance. This is accomplished by capping the ampule with an ampule cap. If the ampule is securely capped, the ampule should be safe from contamination for several hours. The absorbance will decrease by approximately 3% during the first hour and will not change significantly afterwards.

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested may change with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time, and other factors. A single dissolved oxygen test rarely reflects the accurate over-all condition of a body of water. Several samples taken at different times, locations, and depths are recommended for most reliable results. Samples must be tested immediately upon collection although only a small error results if the absorbance reading is taken several hours later.

Accuracy Check

The results of this procedure may be compared with the results of a titrimetric procedure (request Lit. Code 8042) or *sens**ion***™*6* Dissolved Oxygen Meter (Cat. No. 51850-01).

Method Performance

Precision

Standard: 36.0 mg/L O_2

Program	95% Confidence Limits of Distribution
448	33.3–38.7 mg/L O_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Concentration	ΔAbs	ΔConcentration
5 mg/L	0.010	0.34 mg/L O_2
20 mg/L	0.010	0.44 mg/L O_2
40 mg/L	0.010	0.64 mg/L O_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The High Range Dissolved Oxygen AccuVac Ampul contains reagent vacuum sealed in a 12-mL ampule. When the AccuVac Ampul is broken open in a sample containing dissolved oxygen, it forms a yellow color which turns purple. The purple color development is proportional to the concentration of dissolved oxygen. Test results are measured at 680 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
High Range Dissolved Oxygen AccuVac®Ampuls, 0–10 mg/L with 2 reusable ampule caps	1 ampul	25/pkg	25150-25

Required Apparatus

Polypropylene Beaker, 50-mL, Low Form, with pour spout		each	1080-41
Sample Cells, 10-mL, w/cap	1	6/pkg	24276-06

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3/04 1ed

Oxygen Scavengers

Method 8140

Powder Pillows

Iron Reduction Method for Oxygen Scavengers

5 to 600 µg/L carbohydrazide;
3 to 450 µg/L DEHA;
9 to 1000 µg/L hydroquinone;
13 to 1500 µg/L iso-ascorbic acid [ISA];
15 to 1000 µg/L methylethyl ketoxime [MEKO]

Scope and Application: For testing residual corrosion inhibitors (oxygen scavengers) in boiler feed water or condensate

Tips and Techniques

- Temperature and time affect results. Be sure to control these factors as described below.
- Analyze samples immediately. Do not preserve for later analysis.
- The sample temperature should be 25 ±3 °C (77 ±5 °F).
- Soak glassware with 1:1 hydrochloric acid solution. Rinse several times with deionized water. These two steps will remove iron deposits that can cause slightly high results.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- To determine ferrous iron concentration, repeat the procedure, but do not add DEHA Reagent 2 (step 5). Correct for the ferrous iron concentration by touching **Options** and **Reagent Blank**. Touch **On**. The reading attributed to the ferrous iron concentration will appear. Touch **OK**.

Powder Pillows — Method 8140

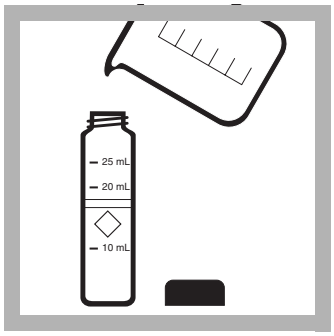
1. Touch

Hach Programs.

Select from the following programs:

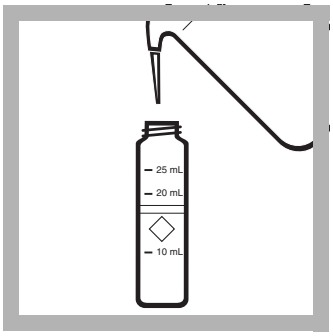
180 O Scav-Carbohy
181 O Scav-DEHA
182 O Scav-Hydro.
183 O Scav-ISA
184 O Scav-MEKO

Touch **Start**.

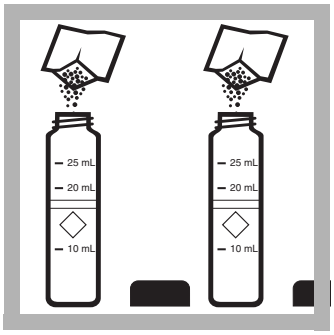


2. Fill a round sample cell with 25 mL of sample (this is the prepared sample).

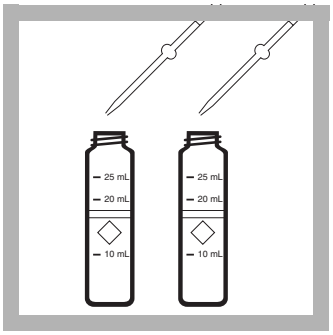
When determining oxygen scavengers that react quickly with oxygen at room temperature, cap the sample cell.



3. Fill a second sample cell with 25 mL of deionized water (this is the blank).

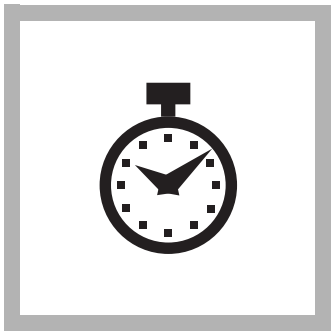


4. Add the contents of one DEHA Reagent 1 Powder Pillow to each sample cell. Swirl to mix.



5. Add 0.5 mL of DEHA Reagent 2 Solution to each sample cell. Mix. Place both sample cells in the dark.

A purple color will develop if an oxygen scavenger is present.



6. Touch the timer icon.

Touch **OK**.

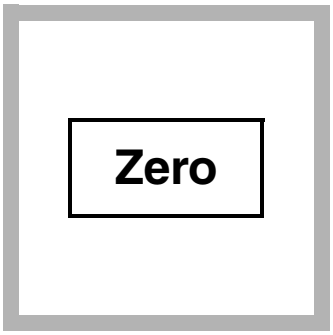
A ten-minute reaction period (or a two-minute reaction period for hydroquinone) will begin.

Keep the sample cells in the dark during the reaction period.

7. When the timer beeps, wipe the blank and place it into the cell holder.

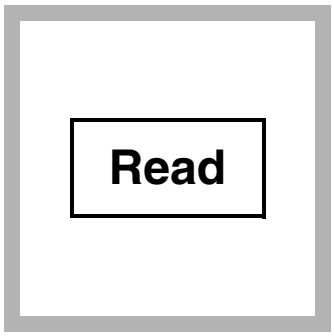
Close the cover.

Note: *For greater accuracy, read the MEKO result immediately after the timer beeps.*



8. Touch **Zero**.

9. Immediately wipe the prepared sample and place it into the cell holder.



10. Touch **Read.**

Results will appear in µg/L.

Interferences

Interfering Substance	Interference Levels and Treatments
Borate (as $Na_2B_4O_7$)	Greater than 500 mg/L
Cobalt	Greater than 0.025 mg/L
Copper	Greater than 8.0 mg/L
Ferrous Iron	All levels
Hardness (as $CaCO_3$)	Greater than 1000 mg/L
Light	Light may interfere. Keep sample cells in the dark during color development.
Lignosulfonates	Greater than 0.05 mg/L
Manganese	Greater than 0.8 mg/L
Molybdenum	Greater than 80 mg/L
Nickel	Greater than 0.8 mg/L
Phosphate	Greater than 10 mg/L
Phosphonates	Greater than 10 mg/L
Sulfate	Greater than 1000 mg/L
Temperature	Sample temperatures below 22 °C or above 28 °C (72 °F or 82 °F) may affect test accuracy.
Zinc	Greater than 50 mg/L

Substances which reduce ferric iron will interfere. Substances which complex iron strongly may also interfere.

Sample Collection, Preservation, and Storage

Collect samples in clean, dry, plastic or glass containers. Avoid excessive agitation or exposure to sunlight when sampling. Rinse the container several times with the sample. Allow the container to overflow and cap the container so that there is no headspace above the sample. Rinse the sample cell several times with sample, then carefully fill to the 25-mL mark. Perform the analysis immediately.

Method Performance

Precision

Program	Standard Level	95% Confidence Limits of Distribution
180	375 µg/L	370–380 µg/L
181	280 µg/L	276–284 µg/L
182	750 µg/L	740–760 µg/L
183	1100 µg/L	1085–1115 µg/L
184	750 µg/L	735–765 µg/L

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	ΔAbs	ΔConcentration
180	0.010	5 µg/L
181	0.010	3 µg/L
182	0.010	9 µg/L
183	0.010	13 µg/L
184	0.010	15 µg/L

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Diethylhydroxylamine (DEHA) or other oxygen scavengers present in the sample react with ferric iron in DEHA Reagent 2 Solution to produce ferrous ion in an amount equivalent to the DEHA concentration. This solution then reacts with DEHA 1 Reagent, which forms a purple color with ferrous iron proportional to the concentration of oxygen scavenger. Test results are measured at 562 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Oxygen Scavenger Reagent Set (50 tests)			24466-00
Includes:			
(2) DEHA Reagent 1 Powder Pillows	2 pillows	100/pkg	21679-69
(1) DEHA Reagent 2 Solution	1 mL	500 mL	21680-49
Hydrochloric Acid, 1:1 (6.0 N)		500 mL	884-49
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Dropper, 0.5 and 1.0-mL marks	1	20/pkg	21247-20
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 8311 **Indigo Method**

AccuVac® Ampul
Scope and Application: For water

LR (0.01 to 0.25 mg/L O_3),
MR (0.01 to 0.75 mg/L O_3),
HR (0.01 to 1.50 mg/L O_3)

Tips and Techniques

- Analyze sample immediately. Do not preserve for later analysis.
- Use tap water or deionized water for the blank (ozone-free water).
- The sequence of measuring the blank and the sample is reversed in this procedure.

AccuVac Ampul Method 8311

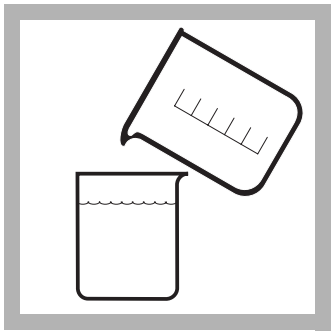
1. Touch

Hach Programs.

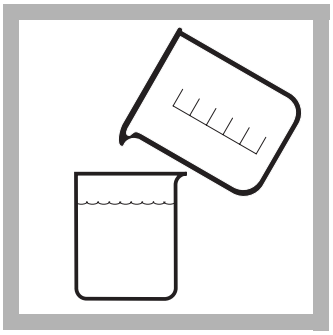
Select from the following programs:

454 Ozone LR AV
455 Ozone MR AV
456 Ozone HR AV

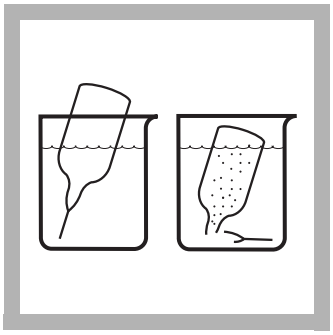
Touch **Start**.



2. Gently collect at least 40 mL of sample in a 50-mL beaker.

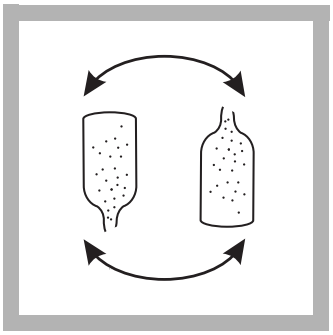


3. Collect at least 40 mL of ozone-free water (the blank) in another 50-mL beaker.



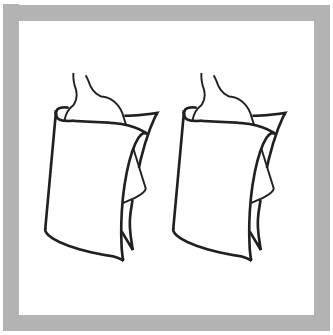
4. Fill one Indigo Ozone Reagent AccuVac Ampul with the sample and another with the blank.

Keep the tip immersed while the ampule fills.



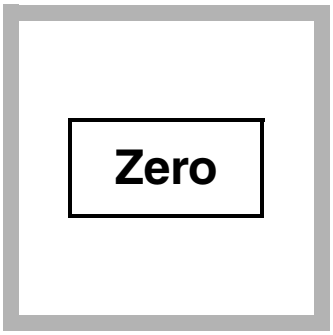
5. Quickly invert both ampules several times to mix.

Some of the blue color will be bleached if ozone is present.



6. Wipe the ampules with a cloth to remove fingerprints or other marks.

7. Place the sample into the cell holder.

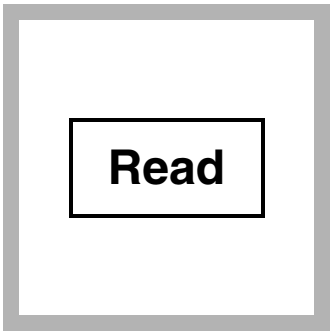


8. Touch **Zero**.

The display will show:

0.00 mg/L O_3

9. Place the blank into the cell holder.



10. Touch **Read.**

Results will appear in mg/L O_3.

Sample Collection, Storage, and Preservation

The chief consideration when collecting a sample is to prevent the escape of ozone from the sample. The sample should be collected gently and analyzed immediately. Warming the sample, or disturbing the sample by stirring or shaking, will result in ozone loss. After collecting the sample, do not transfer it from one container to another unless absolutely necessary.

Stability of Indigo Reagent

Because indigo is light-sensitive, the AccuVac Ampuls should be kept in the dark at all times. The indigo solution, however, decomposes slowly under room light after filling with sample. The blank ampule can be used for multiple measurements during the same day.

Method Performance

Precision

Standard:

0.15 mg/L for program number 454
0.45 mg/L for program number 455
1.00 mg/L for program number 456

Program	95% Confidence Limits of Distribution
454	0.14–0.16 mg/L O_3
455	0.43–0.47 mg/L O_3
456	0.97–1.03 mg/L O_3

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	ΔAbs	ΔConcentration
454	0.010	0.01 mg/L O_3
455	0.010	0.01 mg/L O_3
456	0.010	0.01 mg/L O_3

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The reagent formulation adjusts the sample pH to 2.5 after the ampule has filled. The indigo reagent reacts immediately and quantitatively with ozone. The blue color of indigo is bleached in proportion to the amount of ozone present in the sample. Other reagents in the formulation prevent chlorine interference. No transfer of sample is needed in the procedure. Therefore, ozone loss due to sampling is eliminated. Test results are measured at 600 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Select one or more Ozone AccuVac® Ampuls based on range:			
0–0.25 mg/L	2 ampuls	25/pkg	25160-25
0–0.75 mg/L	2 ampuls	25/pkg	25170-25
0–1.50 mg/L	2 ampuls	25/pkg	25180-25

Required Apparatus

Polypropylene Beaker, 50-mL, Low Form, with pour spout	each	1080-41
Snapper, AccuVac®	each	24052-00

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

PCB (Polychlorinated Biphenyls)

Method 10050 **Immunoassay Method***

Scope and Application: For soil

* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

This method analyzes for PCB that has been extracted from soil samples. Sample extracts, calibrators, and reagents are added to cuvettes coated with PCB-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 20 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously.

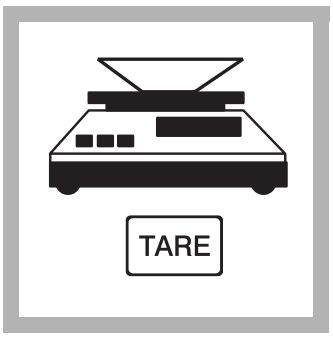
Tips and Techniques

- Read the entire procedure before starting. Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis. A 1-cm square cell holder is required.
- Timing is critical; follow instructions carefully.
- A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack* on page *7*. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator or sample. Antibody Cuvettes are not reusable.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- There are two protocols in this procedure, one for levels of 1 ppm and 5 ppm, and another for 10 ppm and 50 ppm. Each uses a different quantity of calibrator and sample extract as follows:

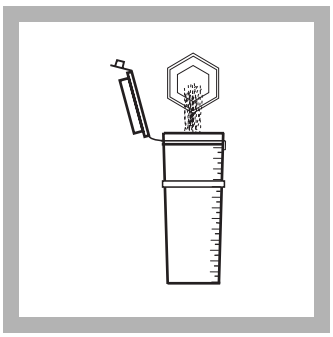
Range (as Arochlor 1248)	Volume of calibrator and sample extract used
1 ppm and 5 ppm	50 µL
10 ppm and 50 ppm	10 µL

- To test across ranges, such as 1 and 50 ppm, test the lower concentration first. If the result is positive then test at the higher level. If the result of the test at the lower concentration is negative, the higher range test will be negative also, and need not be performed.
- The same filtered extract can be used for both protocols if it is tightly capped between assays. The maximum time between assays cannot exceed one-half hour.
- Store the reagents at 4 °C when they are not in use. Allow the reagents to reach room temperature before using them in an analysis. Actual testing may be done at temperatures ranging from 1– 38 °C.
- The Soil Extractant contains methyl alcohol which is poisonous and flammable. Before using this and other reagents, read the Material Safety Data Sheet (MSDS) for proper use of protective equipment and other safety information.
- Hach Company recommends wearing protective nitrile gloves for this procedure.

Soil Extraction Procedure



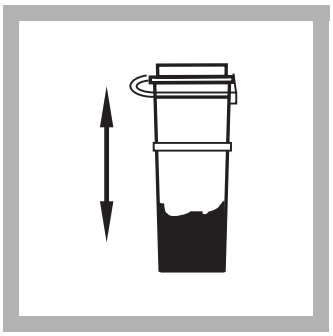
1. Weigh out 5 g of soil in the plastic weighing boat.



2. Carefully pour the soil into an extraction vial.

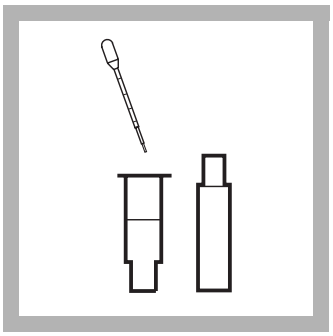
3. Use the 5-gram scoop to add one scoop of sodium sulfate to the extraction vial.

4. Use the graduated cylinder to transfer 10 mL of Soil Extractant into the extraction vial.



5. Cap the extraction vial tightly and shake vigorously for one minute.

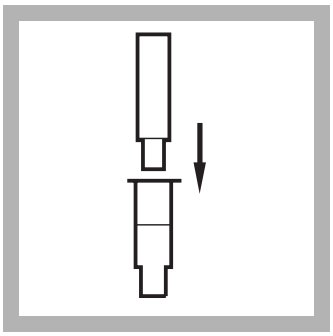
6. Allow to settle for at least one minute. Carefully open the extraction vial.



7. Using the disposable bulb pipet, withdraw 1.0–1.5 mL from the liquid layer at the top of the extraction vial.

Transfer it into the filtration barrel (the bottom part of the filtering assembly into which the plunger inserts).

Note: *Do not use more than 1.5 mL. The bulb is marked in 0.25-mL increments.*

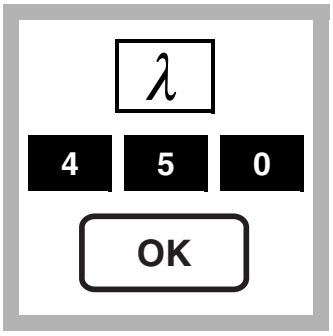


8. Insert the filtration plunger into the filtration barrel. Press firmly on the plunger until the sample extract is forced upward into the center of the plunger.

Use the resultant filtrate for the immunoassay in the *Immunoassay Procedure for Soil Extracts* on page 3.

Note: *It may be necessary to place the filtration assembly on a table and press down on the plunger.*

Immunoassay Procedure for Soil Extracts

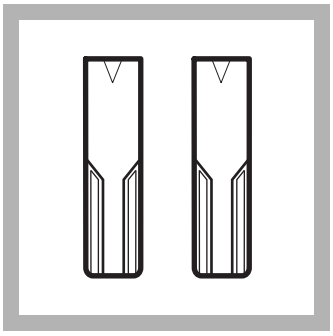


1. Touch

Single Wavelength

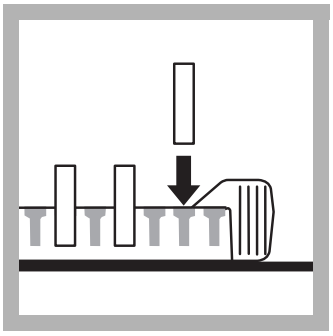
then touch the λ button. Type in 450 nm and touch **OK.**

Note: *Use the 1-cm square cell holder for this procedure.*

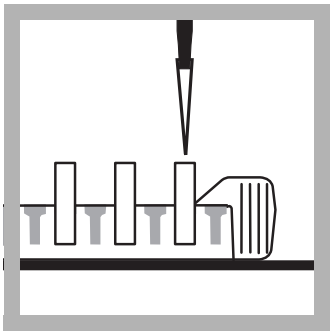


2. Label an Antibody Cuvette for each calibrator and each sample to be tested.

Note: *As many as 10 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.*



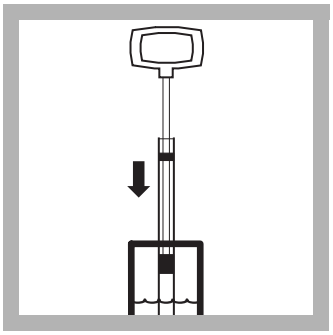
3. Place the cuvettes snugly into the rack.



4. Pipet 0.5 mL of Diluent Solution into each cuvette.

Note: *The same pipette tip can be used repeatedly for this step.*

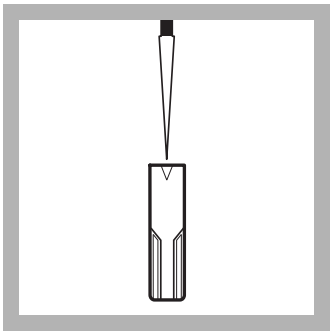
Note: *Have the necessary apparatus at hand for the next four steps as they must be done without delay.*



5. Use a Wiretrol® pipet to transfer the appropriate volume of calibrator or sample extract into each cuvette.

Note: *When testing at the 1 ppm and/or 5 ppm levels, use 50 µL of calibrator and sample. When testing at the 10 ppm and/or 50 ppm levels, use 10 µL of calibrator and sample.*

Note: *Use a separate capillary tube for each solution.*

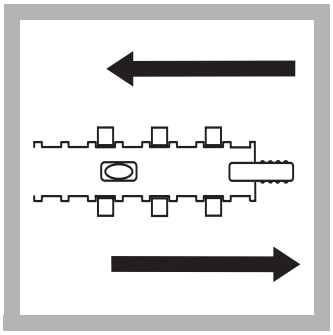


6. Immediately pipet 0.5 mL of PCB Enzyme Conjugate into each calibrator and sample cuvette.

Note: *The same pipette tip can be used repeatedly for this step.*

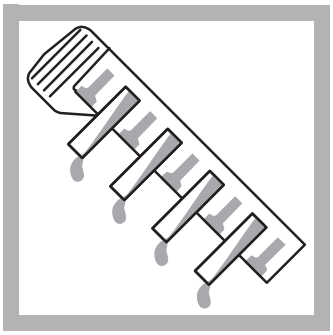
7. Touch the timer icon. Enter 10 minutes and touch **OK.**

A 10-minute reaction time will begin. Proceed immediately to the next step.

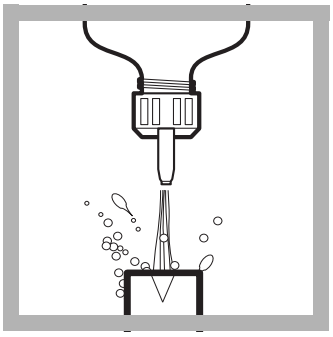


8. Mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack* on page 7.

9. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.



10. At the end of the 10-minute period, discard the contents of all the cuvettes into an appropriate waste container.

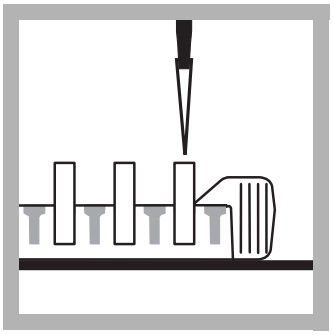


11. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.

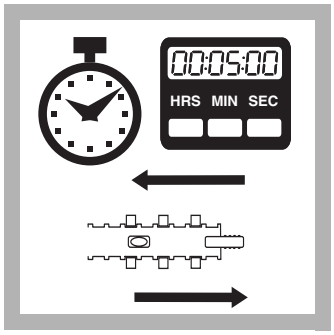
Color Development

Note: *Timing is critical; follow instructions carefully*



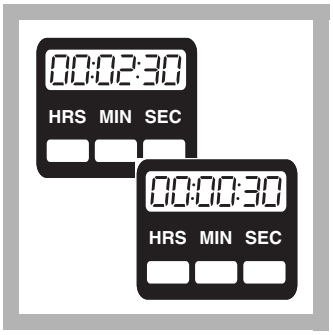
12. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: *Use a new pipette tip for each cuvette.*

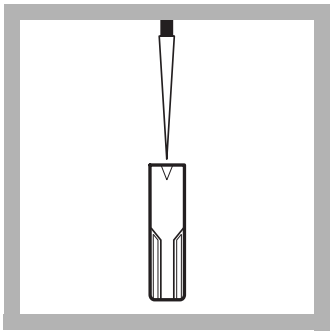


13. Touch the timer icon. Enter 5 minutes. Touch **OK**.

A 5-minute reaction period will begin. Mix following the instructions in *Using the 1-cm MicroCuvette Rack*.



14. After 2.5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.



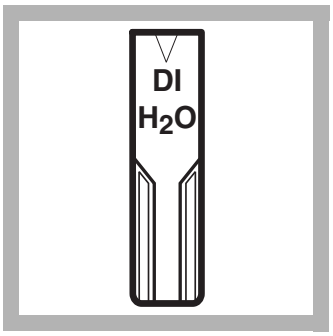
15. At the end of the 5-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in *step 12*.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack* on page *7*.

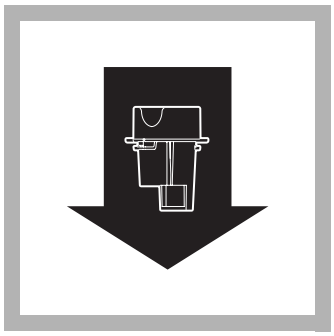
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

Note: *The same pipette tip can be used repeatedly for this step.*

Measuring the Color



16. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

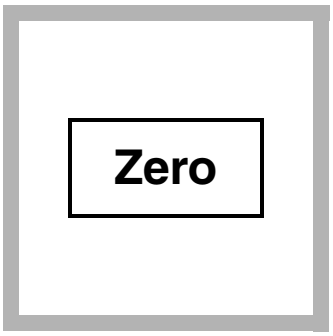


17. Install the 1-cm square cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Place the filled zeroing cuvette into the cell holder—arrow pointing towards the left side of the instrument.

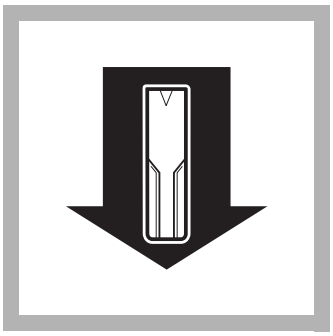
Orient the arrow in the same direction for all cuvettes.



18. Touch **Zero**.

The display will show:

0.000 Abs

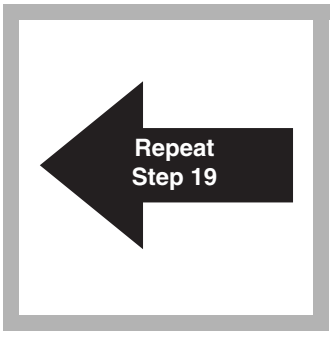


19. Place the first calibrator into the cell holder.

Touch **Read.**

Results in Abs will appear.

The display will give an absorbance reading. Record the results for each calibrator and sample.



20. Repeat *step 19* for all remaining calibrators and samples.

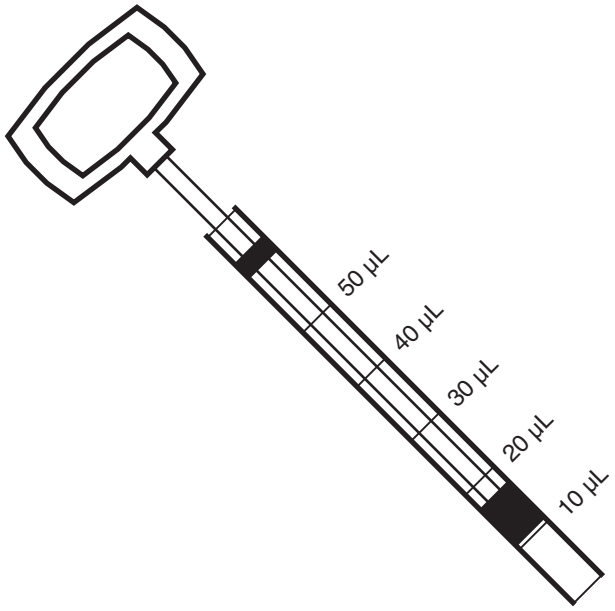
See *Interpreting and Reporting Results* on page *8* for help with interpretation of results.

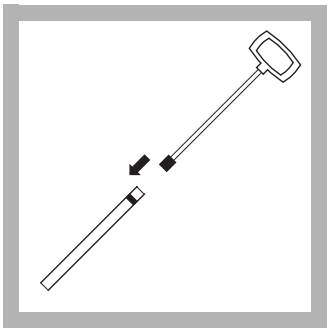
Using the WireTrol Pipet

The Wiretrol Pipet can accurately measure small quantities of liquids. It consists of two parts: a Teflon®-tipped plunger and a calibrated capillary tube. Use *Figure 1* to determine the quantity measured at each line on the capillary tube.

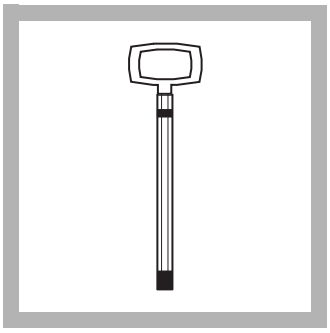
The plunger can be re-used; the capillary tubes must be discarded after one use.

Figure 1 Wiretrol Pipet

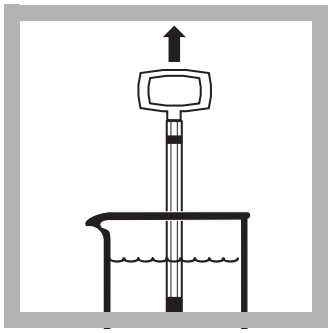




1. Wet the orange Teflon® tip of the Wiretrol plunger in the sample and carefully insert it into the end of the capillary tube with the colored band on it.

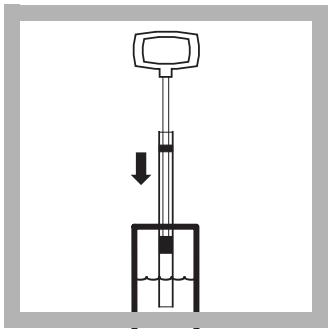


2. Push the tip to the other end of the capillary tube until it barely extends beyond the end of the capillary tube.



3. Submerge the capillary tube below the surface of the liquid to be pipetted. Slowly and smoothly draw the Wiretrol plunger up until the bottom of the plunger tips reaches the appropriate volume line.

Note: *Touch the end of the tube to the side of the vessel to release remaining drops on the capillary tube tip.*

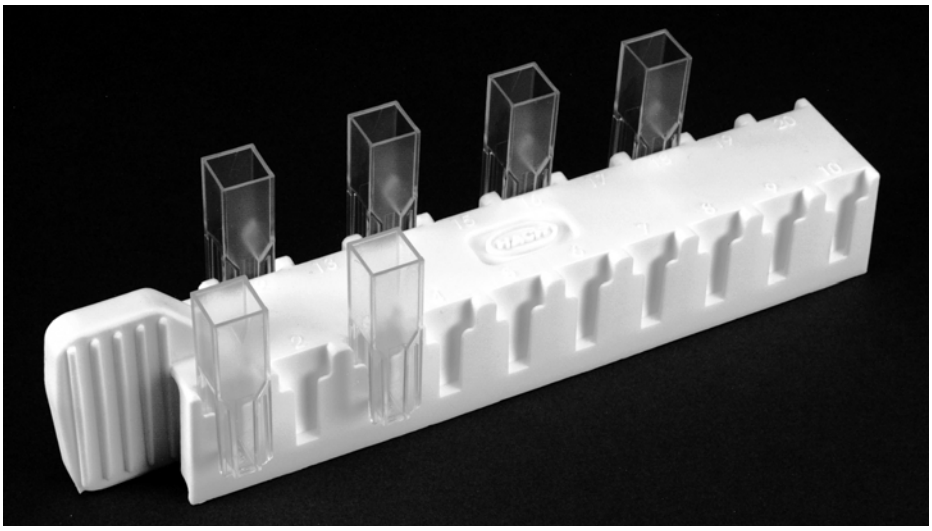


4. To discharge the pipet, place the tip of the capillary tube below the surface of the solution and push the Wiretrol plunger down in one smooth motion. Change capillary tubes for each calibrator and sample.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 2*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 2 The 1-cm MicroCuvette Rack



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of PCB and the reading. In other words, the higher the reading, the lower the concentration of PCB.

If the sample reading is…	the sample PCB Concentration is…
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example

Readings:

1 ppm PCB Calibrator: 0.775 Abs

5 ppm PCB Calibrator: 0.430 Abs

Sample #1: 0.200 Abs

Sample #2: 0.600 Abs

Sample #3: 0.900 Abs

Interpretation

Interpretation for a soil sample:

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of PCB is greater than both 1 ppm and 5 ppm as Aroclor 1248.

Sample #2 — Sample reading is between the readings for the 1 ppm and 5 ppm PCB calibrators. Therefore the sample concentration of PCB is between 1 ppm and 5 ppm as Aroclor 1248.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of PCB is less than both 5 ppm and 1 ppm as Aroclor 1248.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the PCB Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The PCB immunoassay cannot differentiate between the various Aroclors, but it detects their presence in differing degrees.

Table 1 Various PCBs in Soil

Compound	Concentration (ppm) to give a positive result at			
	1 ppm	5 ppm	10 ppm	50 ppm
1248	1	5	10	50
1016	2	9	20	67
1242	1.2	6	14	50
1254	1.4	4.6	11	28
1260	1.1	4.9	11	38

The following compounds are not detectable at 1000 ppm.

Biphenyl	2,4,6-trichlorophenyl	1,3-dichlorobenzene
2,4-dicholorophenyl	pentachlorophenol	1,4-dichlorobenzene
2,4,5-trichlorphenyl	1,2-dichlorobenzene	1,2,4-trichlorobenzene

Sample Collection and Storage

Analyze the samples as soon as possible after collection. If the samples must be stored, collect them in glass or Teflon® containers that have been washed with soap and water and rinsed with methanol. The container should be capped with a Teflon-lined cap. If a Teflon cap is not available, aluminum foil rinsed in methanol may be used as a substitute cap liner.

Summary of Method

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Antibodies specific for PCB are attached to the walls of plastic cuvettes. They selectively bind and remove PCB from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and PCB compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by PCB and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of PCB in the sample. The resulting color is then compared with a calibrator to determine whether the PCB concentration in the sample is greater or less than the threshold levels. The PCB concentration is inversely proportional to the color development: the lighter the color, the higher the PCB concentration. Test results are measured at 450 nm.

Required Reagents

Description	Unit	Cat. No.
Reagent Set, PCB*	20 cuvettes	27735-00
Deionized water	500 mL	272-48

Required Apparatus

Cell holder, 1-cm square	each	59065-00
Caps, flip spout	2/pkg	25818-02
Marker, laboratory	each	20920-00
Rack, for 1-cm Micro Cuvettes	each	48799-00
TenSette®, Pipet, 0.1–1.0 mL	each	19700-01
Tips, for TenSette®, Pipet 19000-01	1000/pkg	21856-28
Wipes, disposable	box	20970-00

For Soil Extraction only:

Soil Scoop, 5-g, 4.25-cc	each	26572-05
Soil Extraction Refill Kit	each	27752-00
Includes:		
Dropper, LDPE, 0.5 and 1.0-mL	20/pkg	21247-20
Filter and Barrel Assembly	20/pkg	25676-20
Sodium Sulfate, anhydrous	250 g	7099-29
Soil Extractant Solution	200 mL	25677-29
Soil Sample Container	20/pkg	25929-20
Weighing Boat, 8.9-cm square	20/pkg	21790-20
Spatula, disposable	2/pkg	25693-20

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.

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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

★Method 8047 4-Aminoantipyrine Method* (0.002 to 0.200 mg/L)

Scope and Application: For water and wastewater; USEPA accepted (distillation required)**

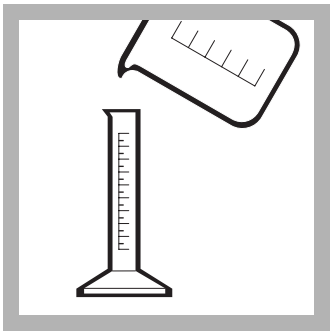
* Adapted from *Standard Methods for the Examination of Water and Wastewater*
** Procedure is equivalent to USEPA method 420.1 for wastewater

Tips and Techniques

- Analyze samples within four hours to avoid oxidation.
- Spilled reagent affects test results and is hazardous to skin and other materials.
- Use chloroform only with proper ventilation.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Phenol 2 Reagent Powder Pillows contain potassium ferricyanide. Both chloroform (D022) and cyanide (D001) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal as a reactive waste. Be sure that cyanide solutions are stored in a caustic solution with a pH >11 to prevent release of hydrogen cyanide gas. See *Waste Management and Safety* on page *55* for further information on proper disposal of these materials.

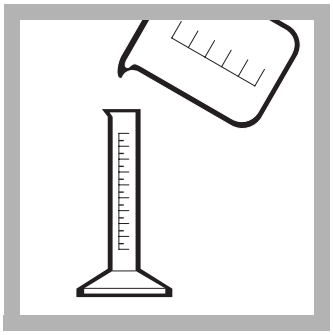
Method 8047

1. Touch **Hach Programs**.
Select program **470 Phenols**.
Touch **Start**.



2. Measure 300 mL of deionized water in a 500-mL graduated cylinder.

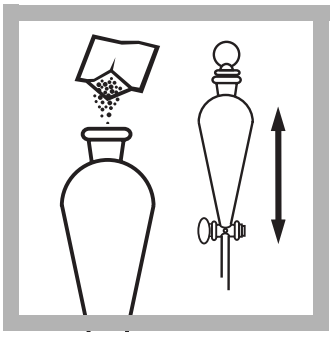
3. Pour the measured deionized water into a 500-mL separatory funnel (this is the blank).



4. Measure 300 mL of sample in a 500-mL graduated cylinder.

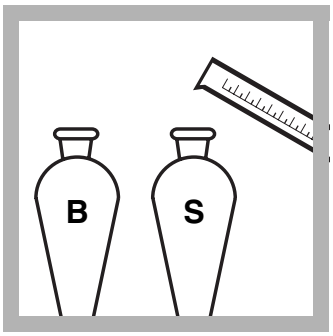
5. Pour the measured sample into another 500-mL separatory funnel (this is the prepared sample).

6. Add 5 mL of Hardness Buffer to each separatory funnel. Stopper and shake to mix.

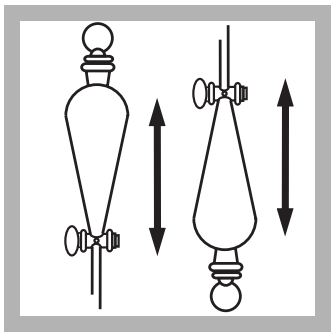


7. Add the contents of one Phenol Reagent Powder Pillow to each separatory funnel. Stopper and shake to dissolve.

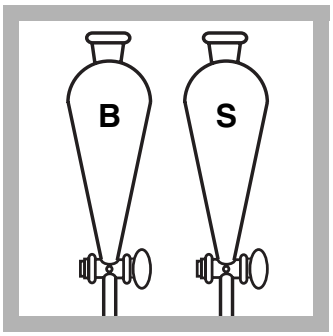
8. Add the contents of one Phenol 2 Reagent Powder Pillow to each separatory funnel. Stopper and shake to dissolve.



9. Add 30 mL of chloroform to each separatory funnel. Stopper each funnel.

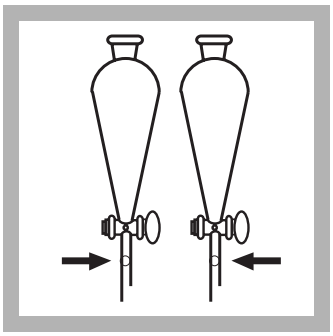


10. Invert each funnel and temporarily vent. Shake each funnel briefly and vent. Then vigorously shake each funnel for a total of 30 seconds (venting if necessary).



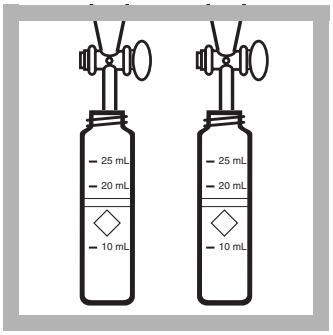
11. Remove the stoppers. Allow both funnels to stand until the chloroform settles to the bottom of the funnel.

The chloroform layer will be yellow to amber if phenol is present.



12. Insert a large, pea-sized cotton plug into the delivery tube of each funnel.

Filtering the chloroform layer through the cotton removes suspended water or particles. The volume of chloroform extract will be about 25 mL.

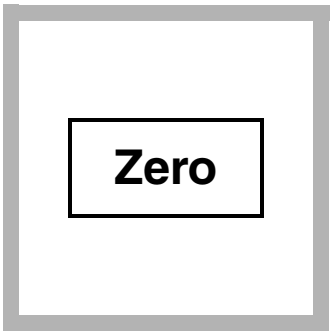


13. Drain the chloroform layers into separate sample cells (one for the blank and one for each sample).

Proceed promptly through the rest of the procedure because the chloroform will evaporate and cause high readings.

The water phase contains chloroform, which is hazardous. Dispose of properly.

14. Wipe the blank and place it into the cell holder.



15. Touch **Zero**.

The display will show:

0.000 mg/L Phenol

16. Wipe the prepared sample and place it into the cell holder.

Touch **Read.**

Results will appear in mg/L Phenol.

Interferences

Interfering Substance	Interference Levels and Treatments
pH	The sample pH must be between 3 and 11.5 for best results.
Oxidizing or reducing agents	May interfere. Distill samples (see procedure below).
Sulfides or suspended matter	Distillation or the following pretreatment is necessary: **1.** Fill a clean 500-mL graduated cylinder with 350 mL of sample. Pour the sample into a clean 500-mL Erlenmeyer flask. **2.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow (Cat. No. 2418-99). Swirl to mix. **3.** Filter 300 mL of the sample through a folded filter paper (Cat. No. 1894-57). Use this solution in *step 5*.

Sample Collection, Storage and Preservation

Most reliable results are obtained when samples are analyzed within four hours after collection. Use the following storage instructions only if prompt analysis is not possible. Collect 500 mL of sample in clean glass containers and add the contents of two Copper Sulfate Powder Pillows (Cat. No. 14818-66). Adjust the pH to 4 or less with 10% Phosphoric Acid Solution (Cat. No. 14769-32). Store at 4 °C (39 °F) or lower and analyze within 24 hours.

Accuracy Check

Standard Solution Method

For greater accuracy, analyze standard solutions when new lots of reagent are first used.

1. Weigh out 1.00 g of Phenol, ACS. Transfer to a 1000-mL volumetric flask. Dilute to the mark with freshly boiled and cooled deionized water. This is a 1000-mg/L stock solution.
2. Pipet 10.0 mL of the 1000-mg/L stock solution to a 1000-mL volumetric flask. Dilute to the mark with deionized water. This is a 10-mg/L working solution.
3. Prepare a 0.200-mg/L standard solution by pipetting 10.0 mL of the working solution into a 500-mL volumetric flask. Dilute to the mark with deionized water.
4. Perform the phenol procedure described above using the prepared standard.
5. To adjust the calibration curve using the reading obtained with the standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
6. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.100 mg/L phenol

Program	95% Confidence Limits of Distribution
470	0.091–0.109 mg/L phenol

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.002 mg/L phenol

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Distillation

This procedure is in the Hach Distillation Apparatus Manual in step-by-step illustrated format.

1. Set up the Hach Distillation Apparatus by assembling the general purpose apparatus as shown in the Distillation Apparatus Manual. Use the 500-mL Erlenmeyer flask to collect the distillate. It may be necessary to use a laboratory jack to elevate the flask.
2. Place a stirring bar into the flask.
3. Measure 300 mL of water sample in a clean 500-mL graduated cylinder. Pour it into the distillation flask.
4. For proof of accuracy, use a 0.200-mg/L phenol standard (see *Accuracy Check* on page *4*) in addition to the sample.
5. Using a serological pipet, add 1 mL of Methyl Orange Indicator to the distillation flask.
6. Turn on the stirrer power switch. Set the stir control to 5.
7. Add 10% Phosphoric Acid Solution drop-wise until the indicator changes from yellow to orange.
8. Add the contents of one Copper Sulfate Powder Pillow and allow to dissolve (omit this step if copper sulfate was used to preserve the sample). Cap the distillation flask.
9. Turn the water on and adjust it so a constant flow is maintained through the condenser. Set the heat control to 10.
10. Collect 275 mL of distillate in the Erlenmeyer flask, then turn the heat off.
11. Fill a 25-mL graduated cylinder to the 25-mL mark with deionized water. Add the water to the distillation flask.
12. Turn the still back on. Heat until another 25 mL of distillate is collected.
13. Using a clean graduated cylinder, re-measure the distillate to make sure 300 mL has been collected. The distillate is ready for analysis.

Summary of Method

The 4-aminoantipyrine method measures all ortho- and meta-substituted phenols. These phenols react with 4-aminoantipyrine in the presence of potassium ferricyanide to form a colored antipyrine dye. The dye is then extracted from the aqueous phase with chloroform and the color is measured at 460 nm. The sensitivity of the method varies with the type of phenolic compound. Because water samples may contain various types of phenolic compounds, the test results are expressed as the equivalent concentration of phenol.

Phenols

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Phenols Reagent Set		100 tests	22439-00
Includes:			
(2) Chloroform, ACS	60 mL	4 L	14458-17
(3) Hardness 1 Buffer Solution, pH 10.1	10 mL	500 mL	424-49
(2) Phenol 2 Reagent Powder Pillows	2	100/pkg	1836-99
(2) Phenol Reagent Powder Pillows	2	100/pkg	872-99
Water, deionized	300 mL	4 liters	272-56

Required Apparatus

Clippers, for opening powder pillows	1	each	968-00
Cotton Balls	1	100/pkg	2572-01
Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 500-mL	1	each	508-49
Funnel, separatory, 500-mL	2	each	520-49
Pipet, volumetric, Class A, 5.00-mL	1	each	14515-37
Ring, support, 4-inch	2	each	580-01
Sample Cells, glass-stoppered, 1-inch, matched pair	1	2/pkg	26126-02
Support, ring stand, 5 x 8 inch base	1	each	563-00

Distillation Reagents and Required Standards

Methyl Orange Indicator Solution, 0.5-g/L	100 mL MDB	148-32
Phenol, ACS	113 g	758-14

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3/04 1ed

Phosphonates

Method 8007 **Persulfate UV Oxidation Method***

Powder Pillows **(0.02 to 2.50 and 1.0 to 125.0 mg/L)**

Scope and Application: For boiler and cooling water, wastewater, and seawater

* Adapted from Blystone, P., Larson, P., *A Rapid Method for Analysis of Phosphate Compounds*, International Water Conference, Pittsburgh, PA. (Oct 26-28, 1981)

Tips and Techniques

- Clean glassware with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49), followed by a distilled water rinse. Do not clean glassware with commercial detergent.
- Wear UV safety goggles while the UV lamp is on.
- Do not handle the UV lamp surface. Fingerprints will etch the glass. Wipe the lamp with a soft, clean tissue between samples.
- The digestion in *step 7* is normally completed in less than 10 minutes. However, contaminated samples or a weak lamp can cause incomplete phosphate conversion. Check conversion efficiency by running a longer digestion and seeing if the readings increase.

Powder Pillows Method 8007

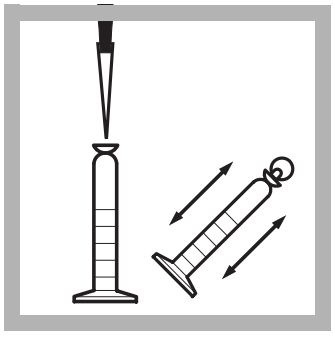
1. Touch

Hach Programs.

Select program

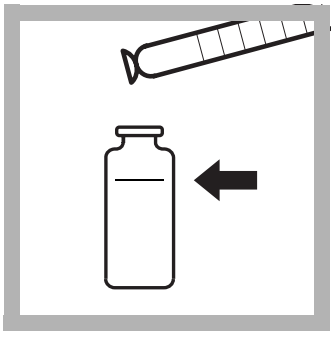
501 Phosphonates.

Touch **Start**.



2. Choose the appropriate sample size from *Table 1* on page *3*. Pipet the chosen volume into a 50-mL graduated cylinder. If necessary, dilute the sample to 50-mL with deionized water and mix well.

3. Fill a round sample cell to the 10-mL mark with diluted sample from *step 2* (this is the blank).

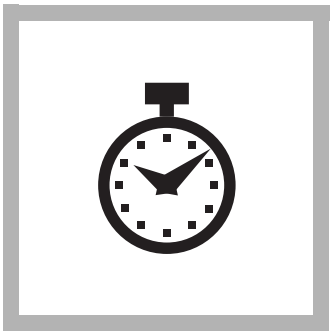


4. Fill a square sample bottle to the 25-mL mark with diluted sample from *step 2*.

5. Add the contents of one Potassium Persulfate for Phosphonate Powder Pillow to the bottle containing 25 mL of sample.

Swirl to mix.

6. Insert the ultraviolet (UV) lamp into the sample bottle.

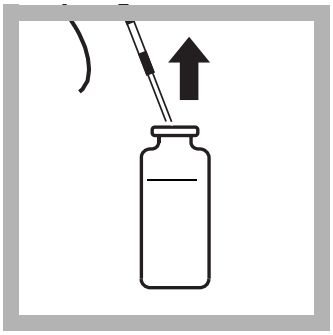


7. Turn on the UV lamp. Touch the timer icon.

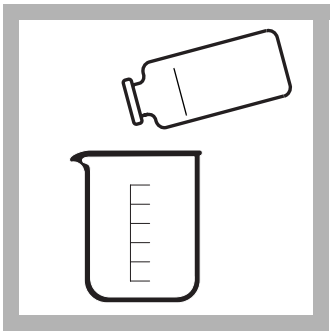
Touch **OK**.

A ten-minute reaction period will begin.

Note: *Phosphonates are converted to orthophosphate in this step.*



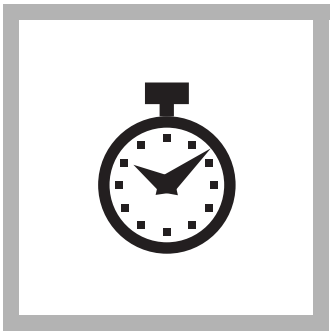
8. When the timer beeps, turn off the UV lamp and remove it from the sample.



9. Pour off about 15 mL of sample into a 50-mL beaker and pour the remaining 10 mL into a round sample cell (this is the prepared sample).

10. Add the contents of one PhosVer 3 Phosphate Reagent Powder Pillow to the blank and prepared sample. Immediately cap and invert to mix.

A blue color will develop if phosphate is present. Both sample and blank cells may develop color. The increase in sample color is proportional to the phosphonate concentration.



11. Touch the timer icon.

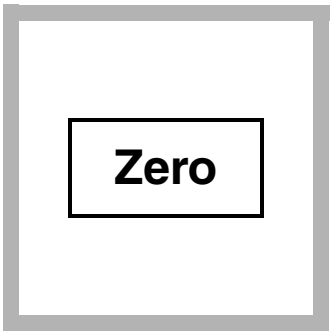
Touch **OK**.

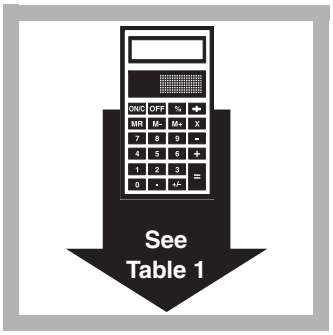
A two-minute reaction period will begin.

If the sample is colder than 15 °C, allow four minutes for color development.

12. When the timer beeps, place the blank into the cell holder.

Complete *steps 13–15* within three minutes after the timer beeps.





13. Touch **Zero**.

The display will show:

0.00 mg/L PO_4^{3-}

14. Wipe the prepared sample and place it into the cell holder.

Touch **Read.**

Results will appear in mg/L PO_4^{3-}.

15. Multiply the value in *step 14* by the appropriate multiplier in *Table 1* to obtain the actual phosphonate concentration.

Complete *steps 13–15* within three minutes after the timer beeps.

Table 1 Expected Ranges with Multipliers

Expected Range (mg/L phosphonate)	Sample Volume (mL)	Multiplier
0–2.5	50	0.1
0–5	25	0.2
0–12.5	10	0.5
0–25	5	1.0
0–125	1	5.0

To express results in terms of active phosphonate, multiply the final value in *step 15* by the appropriate conversion factor in *Table 2*.

Table 2 Conversion Factors by Phosphonate Type

Phosphonate Type	Conversion Factor
PBTC	2.84
NTP	1.050
HEDPA	1.085
EDTMPA	1.148
HMDTMPA	1.295
DETPMPA	1.207
HPA	1.49
active phosphonate (mg/L) = phosphonate concentration from *step 15* × conversion factor	

Interferences

Table 3 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Aluminum	100 mg/L
Arsenate	Interferes at all levels
Benzotriazole	10 mg/L
Bicarbonate	1000 mg/L
Bromide	100 mg/L
Calcium	5000 mg/L
CDTA	100 mg/L
Chloride	5000 mg/L
Chromate	100 mg/L
Copper	100 mg/L
Cyanide	100 mg/L (Increase the UV digestion to 30 minutes.)
Diethanoldithiocarbamate	50 mg/L
EDTA	100 mg/L
Iron	200 mg/L
Nitrate	200 mg/L
NTA	250 mg/L
Orthophosphate	15 mg/L
Phosphites and organophosphorus compounds	React quantitatively. Meta- and polyphosphates do not interfere.
Silica	500 mg/L
Silicate	100 mg/L
Sulfate	2000 mg/L
Sulfide	Interferes at all levels
Sulfite	100 mg/L
Thiourea	10 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

The interference levels will decrease as the sample size increases. For example, copper does not interfere at or below 100 mg/L for a 5.00 mL sample. If the sample volume is increased to 10 mL, copper will begin to interfere above 50 mg/L.

Sample Collection, Storage, and Preservation

Collect samples in acid-cleaned (1:1 HCl, Cat. No. 884-49) plastic or glass bottles that have been rinsed with distilled water. Do not use a commercial detergent. If prompt analysis is impossible, preserve the sample by adjusting to pH 2 or less with Sulfuric Acid (about 2 mL per liter) (Cat. No. 979-49). Store at 4 °C (39 °F). Preserved samples may be stored up to 24 hours. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Ideally, a solution containing the phosphonate product being used should be prepared. This will check the UV conversion of phosphonate to orthophosphate. Alternatively, a phosphate standard can be used to check the accuracy or the colorimetric part of the method.

Standard Solution

A 1-mg/L Phosphate Standard Solution (available from Hach) can be used to check accuracy. Use 10 mL of this standard in place of the prepared sample in step 9. Use deionized water for the blank. A multiplier value from *Table 1* on page *3* is not needed. The result should be 10.0 mg/L phosphate, due to a factor of 10 in calibration.

Method Performance

Precision

Standard: 1.00 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
501	0.97–1.03 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

The sensitivity depends on the sample volume.

Range (mg/L)	Volume (mL)	ΔConcentration
0–2.5	50	0.02 mg/L PO_4^{3-}
0–5	25	0.04 mg/L PO_4^{3-}
0–12.5	10	0.10 mg/L PO_4^{3-}
0–25	5	0.20 mg/L PO_4^{3-}
0–125	1	1.00 mg/L PO_4^{3-}

Sensitivity is expressed as PO_4^{3-} in this table. Use *Table 2* on page *3* to express as a specific phosphonate.

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

This method is directly applicable to boiler and cooling tower samples. The procedure is based on a UV-catalyzed oxidation of phosphonate to orthophosphate. The orthophosphate reacts with the molybdate in the PhosVer 3 reagent to form a mixed phosphate/molybdate complex. This complex is reduced by the ascorbic acid in the PhosVer 3, yielding a blue color that is proportional to the phosphonate present in the original sample. Test results are measured at 880 nm.

Phosphonates

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Phosphonate Reagent Set for 10-mL sample		100 tests	24297-00
Includes:			
PhosVer™ 3 Phosphate Reagent Powder Pillows, 10-mL	2 pillows	100/pkg	21060-69
Potassium Persulfate Powder Pillow for Phosphonate	1 pillow	100/pkg	20847-69
Water, deionized	varies	4 liters	272-56

Required Apparatus

Polypropylene Beaker, 50-mL, Low Form, with pour spout	1	each	1080-41
Cylinder, mixing, graduated, 50-mL	1	each	1896-41
Goggles, UV safety	1	each	21134-00
Pipet, serological, 10-mL	1	each	532-38
Safety bulb	1	each	14651-00
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
UV Lamp with power supply, 115 VAC	1	each	20828-00
or UV Lamp with power supply, 230 VAC	1	each	20828-02

Required Standards

Phosphate Standard Solution, 10-mg/L		946 mL	14204-16

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

DR/2400

Phosphorus, Acid Hydrolyzable

Method 8180 **PhosVer™ 3 with Acid Hydrolysis Method**

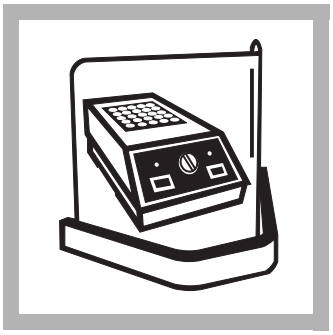
Test 'N Tube™ Vials **(0.06 to 3.50 mg/L PO_4^{3-})**

Scope and Application: For water, wastewater, and seawater

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See *Section 2.6.2 Reagent Blank* on page *20*.
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse with deionized water. Do not use detergents that contain phosphate to clean glassware.
- Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Please see *Waste Management and Safety on page 55* for more information on proper disposal of these materials.

Test 'N Tube Method 8180

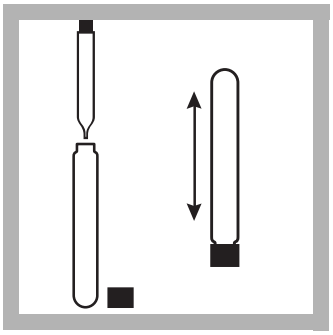


1. Turn on the COD Reactor. Heat to 150 °C. Place the safety shield in front of the reactor.

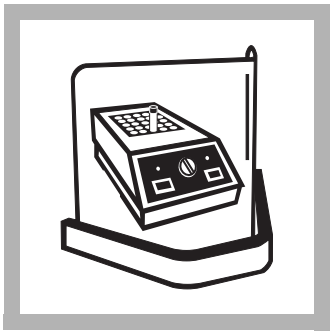
2. Touch **Hach Programs**.

Select program **536 P Total/AH PV TNT**.

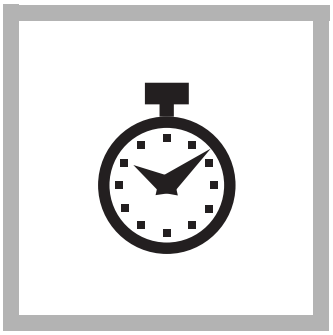
Touch **Start**.



3. Use a TenSette Pipet to add 5 mL of sample to a Total and Acid Hydrolyzable Test Vial. Cap and mix.



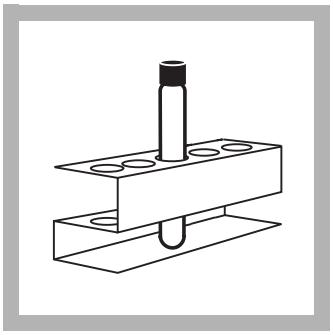
4. Place the vial in the COD Reactor.



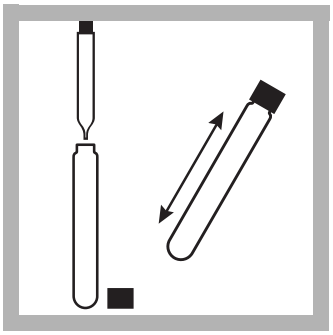
5. Touch the timer icon.

Touch **OK**.

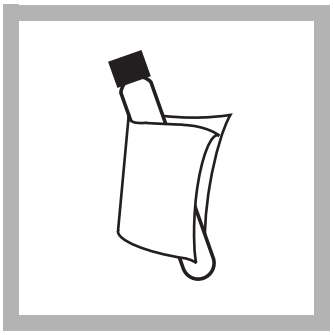
A 30-minute heating period will begin.



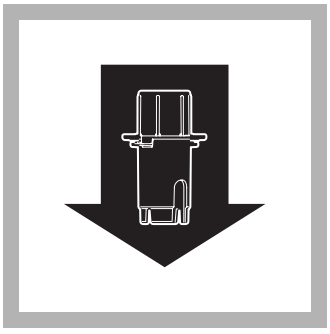
6. After the timer beeps, carefully remove the vial from the reactor. Place it in a test tube rack and cool to room temperature.



7. Using a TenSette Pipet, add 2 mL of 1.00 N sodium hydroxide to the vial. Cap tightly and shake to mix.



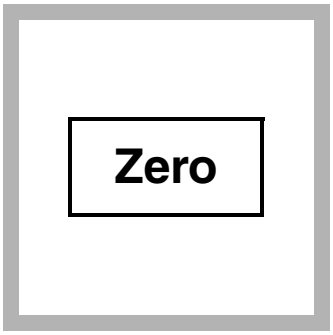
8. Clean the outside of the vial with a towel to remove fingerprints or other marks.



9. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

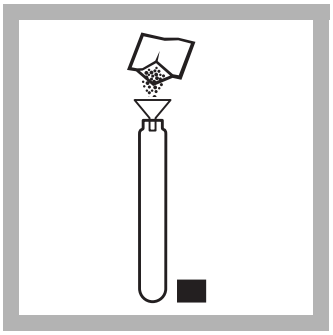
Place the sample vial into the adapter.



10. Touch **Zero**.

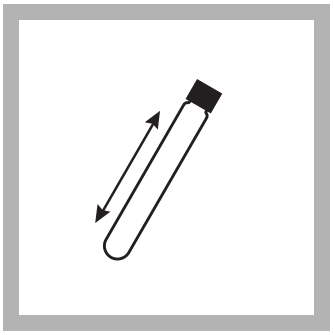
The display will show:

0.00 mg/L PO_4^{3-}

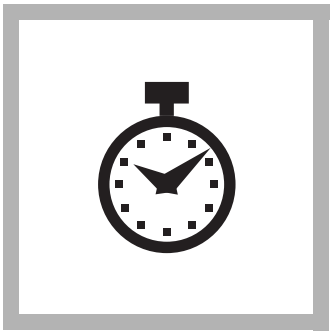


11. Using a funnel, add the contents of one PhosVer 3 Powder Pillow to the vial.

Read results within two to eight minutes after adding the PhosVer 3 reagent.



12. Cap tightly and shake to mix for 10–15 seconds. The powder will not completely dissolve.

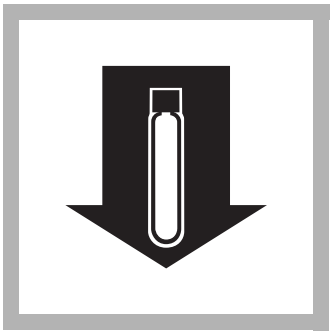


13. Touch the timer icon.

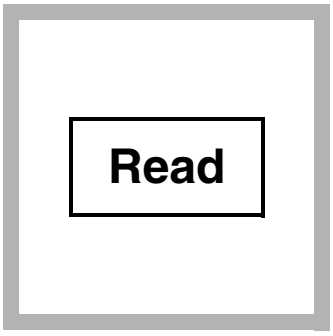
Touch **OK**.

A two-minute reaction period will begin.

14. Clean the outside of the vial with a towel to remove fingerprints or other marks.



15. Wipe the prepared sample and place it into the adapter.



16. Touch **Read.**

Results will appear in mg/L PO_4^{3-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 200 mg/L
Arsenate	All levels
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Sulfide	Greater than 9 mg/L. Remove sulfide interference as follows: **1.** Measure 25 mL of sample into a 50-mL beaker. **2.** Swirling constantly, add Bromine Water (Cat. No. 2211-20) drop-wise until a permanent yellow color appears. **3.** Swirling constantly, add Phenol Solution (Cat. No. 2112-20) drop-wise just until the yellow color disappears. Proceed with step 1.
Turbidity	Large amounts may cause inconsistent results in the test because the acid present in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample Collection, Storage, and Preservation

Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze samples immediately for best results. If prompt analysis is not possible, preserve samples by filtering immediately and storing the sample at 4 °C (39 °F) for up to 48 hours.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Phosphate 2-mL Ampule Standard, 50-mg/L as PO_4^{3-}.

5. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 1.0-mg/L Phosphate Standard Solution in place of the sample. Perform the procedure as described.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 3.00 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
536	2.90–3.10 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.06 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Phosphates present in condensed inorganic forms (meta-, pyro-, or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreating the sample with acid and heat hydrolyzes the condensed inorganic forms to orthophosphate.

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No
Total and Acid Hydrolyzable Phosphorus Reagent Set		50 tests	27427-45
Includes:			
PhosVer 3 Phosphate Reagent Powder Pillows	1 pillow	50/pkg	21060-46
Potassium Persulfate Powder Pillows	1	50/pkg	20847-66
Sodium Hydroxide, 1.54 N		100 mL	27430-42
Sodium Hydroxide Standard Solution, 1.00 N	2 mL	100 mL	1045-42
Total and Acid Hydrolyzable Test Vials*	1 vial	50/pkg	
Water, Deionized		100 mL	272-42

Required Apparatus

COD Reactor, 115/230 VAC, North American plug	1	each	45600-00
COD Reactor, 115/230 VAC, European plug	1	each	45600-02
Funnel, micro	1	each	25843-35
Pipet, volumetric, Class A, 2.00-mL	1	each	14515-36
Pipet, volumetric, Class A, 5.00-mL	1	each	14515-37
Pipet Filler, Safety bulb	1	each	14651-00
Pipet, TenSette, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	1	250/pkg	21997-25
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1–3	each	18641-00

Required Standards

Phosphate Standard Solution, 1-mg/L		500 mL	2569-49
Phosphate Standard Solution, 2-mL Voluette® Ampule, 50-mg/L as PO_4		20/pkg	17120 H

*Not sold separately.

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3/04 1ed

DR/2400

Phosphorus, Acid Hydrolyzable Digestion

★Method 8180 **Acid Digestion Method***

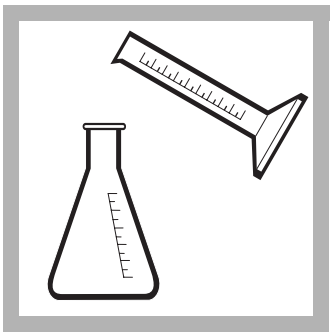
Scope and Application: For water, wastewater, and seawater; USEPA Accepted for wastewater analyses

* Adapted from *Standard Methods for the Examination of Water and Wastewater* 4500-P B & E

Tips and Techniques

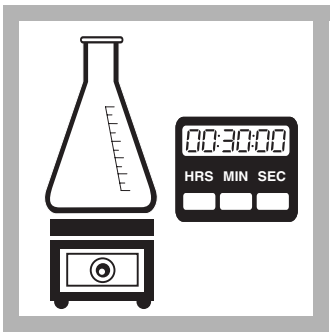
- Rinse all glassware with 1:1 hydrochloric acid. Rinse again with deionized water.
- The results of the reactive phosphorus test after the digestion will include the orthophosphate and the acid-hydrolyzable (condensed) phosphate. The condensed phosphate concentration is determined by subtracting the result of an orthophosphate test from this result. Make sure that both results are in the same units, either mg/L PO_4^{3-} or mg/L P before subtracting. The result from this test is subtracted from the result of a total phosphorus test to determine organic phosphorus.
- For field applications, use a Heatab Cookit Stove (Cat. No. 2206-00) in place of the hot plate.

Powder Pillows Method 8180



1. Use a graduated cylinder to measure 25 mL of sample. Pour the sample into a 125-mL Erlenmeyer flask.

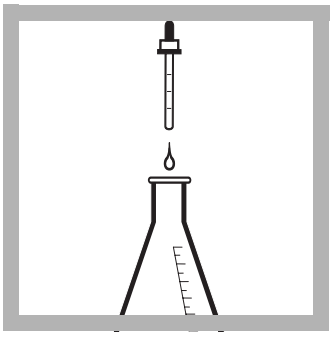
2. Use a 1-mL calibrated dropper to add 2.0 mL of 5.25 N Sulfuric Acid Solution to the flask.



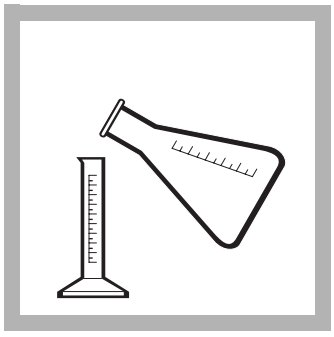
3. Place the flask on a hot plate. Boil gently for 30 minutes.
Do not boil dry.

Concentrate the sample to less than 20 mL for best recovery. After concentration, maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.

4. Cool the sample to room temperature.



5. Use a 1-mL calibrated dropper to add 2.0 mL of 5.0 N Sodium Hydroxide Solution to the flask. Swirl to mix.



6. Pour the sample into a 25-mL graduated cylinder. Adjust the volume to 25 mL with deionized water rinsings from the flask.

7. Proceed with a reactive phosphorus test of the expected acid hydrolyzable phosphorus concentration range. Extend the color development time to 10 minutes for the Ascorbic Acid method.

Interferences

Interfering Substance	Interference Levels and Treatments
Alkaline or highly buffered samples	It may be necessary to add additional acid in step 3 to drop the pH of the solution below 1.
Turbidity	Use 50 mL of sample and double the reagent quantities. Use 25 mL of the reacted sample to zero the instrument in the reactive phosphorus procedure. This compensates for any color or turbidity destroyed by this procedure.

Sample Collection, Storage, and Preservation

Analyze the samples immediately for the most reliable results. If prompt analysis is not possible, samples may be preserved up to 28 days by adjusting the pH to 2 or less with Concentrated Sulfuric Acid (about 2 mL per liter) (Cat. No. 979-49) and storing at 4 °C. Warm the sample to room temperature and neutralize with 5.0 N Sodium Hydroxide (Cat. No. 2450-53) before analysis. Correct for volume additions: see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Summary of Method

Phosphates present in condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat hydrolyzes the condensed inorganic forms to orthophosphate.

This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determining the phosphorus content of the sample. If the ascorbic acid (PhosVer 3) method is used to measure the reactive phosphorus, this method is USEPA accepted for NPDES reporting.

The following reagents and apparatus are required in addition to those required for the active phosphorus test.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Sodium Hydroxide Solution, 5.0 N	2 mL	100 mL MDB	2450-32
Sulfuric Acid Solution, 5.25 N	2 mL	100 mL MDB	2449-32
Water, deionized		4 liters	272-56

Required Apparatus

Cylinder, graduated, 25-mL	1	each	508-40
Flask, Erlenmeyer, 125-mL	1	each	505-43
Hot Plate, 4 inch diameter, 120 VAC	1	each	12067-01
Hot Plate, 4 inch diameter, 240 VAC	1	each	12067-02

Required Apparatus (Field Applications)

Heatab Cookit, with 1 box heatabs	each	2206-00
Heatab Replacements	21/pkg	2207-00

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Phosphorus, Reactive

Method 10055

Ascorbic Acid Rapid Liquid Method*

Pour-Thru Cell

LR (19 to 3,000 µg/L PO_4^{3-})

Scope and Application: For treated and natural waters

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- Clean the Pour-Thru cell and all labware as specified in *Treating Analysis Labware on page 3*.
- Protect the Pour-Thru Cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
- See *Reagent Preparation on page 3* for preparing the Ascorbic Acid reagent.
- Reaction time depends on sample temperature. For most accurate results, samples should be at room temperature (about 20 °C).
- Obtain a reagent blank for each lot of reagent when the phosphate concentration is less than 750 µg/L. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

DR/2400 Pour-Thru Cell — Method 10055

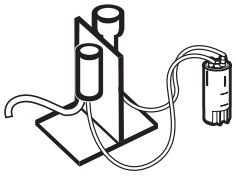
1. Touch

Hach Programs.

Select program

488 P React. LR RL.

Touch **Start**.



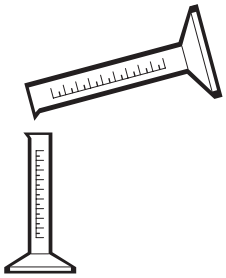
2. Install the Pour-Thru Cell and multi-pathlength cell adapter in the sample cell compartment.

Note: *See Section 2.6.1 on page 26 in the Instrument manual for installation details.*

Flush with 50-mL of deionized water.

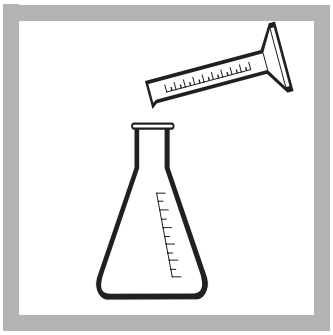
Note: *Use the 25-mm cell pathlength.*

3. Rinse two clean Erlenmeyer flasks three times with the sample.

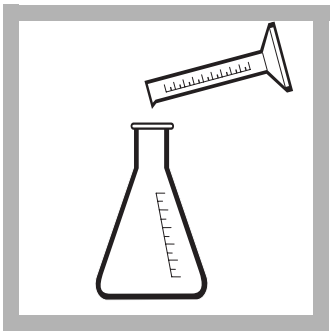


4. Rinse a clean 25-mL plastic graduated cylinder three times with the sample.

5. Fill this rinsed cylinder to the 25-mL mark with sample.



6. Pour the contents of the 25-mL cylinder into one of the flasks.

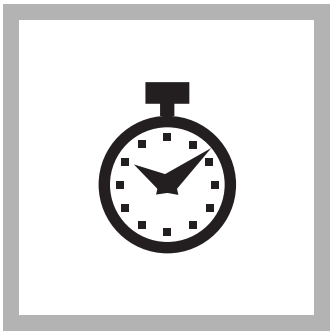


7. Measure a second 25-mL portion of sample into the graduated cylinder and pour the contents into the second flask.

8. Add 1.0 mL of Molybdate reagent to each flask using a Repipet Jr. Dispenser.

Swirl to mix.

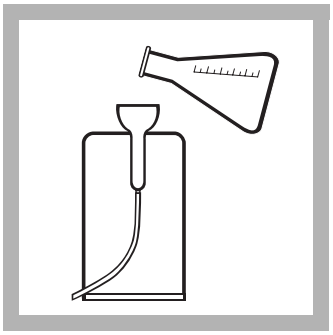
9. Add 1.0 mL of prepared Ascorbic Acid reagent to one of the flasks with a Repipet Jr. Dispenser. Swirl to mix. The remaining flask will be the blank.



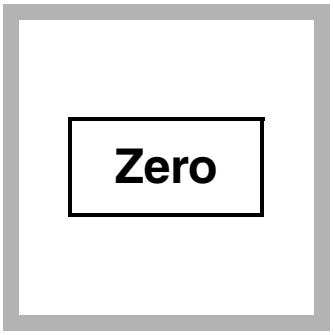
10. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.



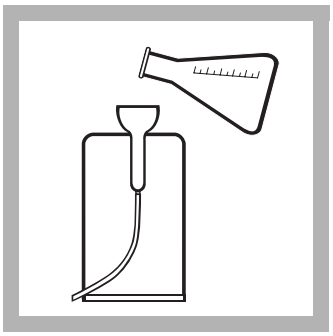
11. When the timer beeps, pour the contents of the flask that contains the blank into the Pour-Thru Cell.



12. After the flow stops, touch **Zero**.

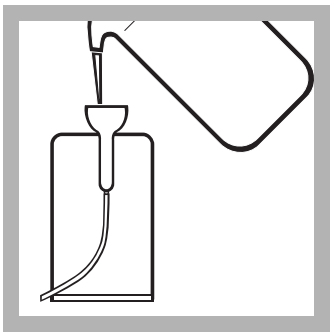
The display will show:

0 µg/L PO_4^{3-}



13. Pour the contents of the second flask into the Pour-Thru Cell.

After the flow stops, touch **Read**. Results will appear in µg/L PO_4^{3-}.



14. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	200 mg/L
Arsenate	Interferes
Chromium	100 mg/L
Copper	10 mg/L
Hydrogen sulfide	Interferes
Iron	100 mg/L
Nickel	300 mg/L
Silica	50 mg/L
Silicate	10 mg/L
Turbidity	Samples with large amounts of turbidity may give inconsistent results because the acid present in the reagents may dissolve some of the suspended particles and variable desorption of orthophosphate from the particles may occur.
Zinc	80 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see *Section 3.3 Interferences* on page *41*.

Treating Analysis Labware

All labware used in this test must be thoroughly cleaned to remove all traces of phosphate. Clean containers with a non-phosphate detergent followed by a rinse with deionized water. Fill and soak for 10 minutes with a 1:25 dilution of Molybdate Reagent in deionized water. Rinse well with deionized water. Keep containers tightly closed when not in use. Treat the Pour-Thru Cell with this same mixture of molybdate and water followed by thorough rinsing with deionized water.

Dedicate these containers for low-level phosphate analysis. If these containers are rinsed and capped after use, only occasional pre-treatment is necessary.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. Remove the color by rinsing with a 1:5 dilution of Ammonium Hydroxide (Cat. No. 106-49), followed by several deionized water rinses. Invert a beaker over the glass funnel of the cell when not in use.

Reagent Preparation

The Ascorbic Acid reagent must be prepared before use. Using a powder funnel, add the contents of one 48 g bottle of Ascorbic Acid Reagent Powder (Cat. No. 26512-55) to one 450 mL bottle of Ascorbic Acid Reagent Dilution Solution (Cat. No. 25999-49). Invert several times and swirl until the powder is completely dissolved.

This solution may develop a yellow color with time but will still give accurate results for up to one month after mixing if stored at 20–25 °C. Record the date of preparation on the bottle and discard any remaining solution after one month. Do not add fresh reagent to previously mixed reagent. Use of this reagent after one month may result in high reagent blanks and low values at high concentrations.

Sampling and Storage

Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use detergents that contain phosphate for cleaning labware.

Analyze samples immediately for best results. If prompt analysis is not possible, preserve samples by filtering immediately and storing at 4 °C (39 °F) for up to 48 hours.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Phosphate Standard Solution Ampule, either 15-mg/L (15,000 µg/L) as PO_4^{3-} or 50-mg/L (50,000 µg/L) as PO_4^{3-}. Use the 15-mg/L standard when the phosphate concentration of samples is less than 1000 µg/L.
5. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Run the test using a 1000-µg/L (1.000-mg/L) phosphate standard solution in place of the sample. Results should be 900 to 1100 µg/L phosphate.
2. To adjust the calibration curve using the reading obtained with the 1000-µg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 1000 µg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
488	976–1024 µg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	19 µg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Reactive phosphorus includes existing orthophosphate in the sample plus a small fraction of condensed phosphate that may be hydrolyzed to orthophosphate during the test. Test results are measured at 880 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Rapid Liquid Low Range Phosphorus Reagent Set			26786-00
Includes:			
Ascorbic Acid Reagent Dilution Solution	1 mL	450 mL	25999-49
Ascorbic Acid Reagent Powder		48 g	26512-55
Molybdate Reagent Solution	2 mL	500 mL	25998-49
Water, deionized		4 L	272-56

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cylinder, graduated, 25-mL, poly	2	each	1081-40
Dispenser, fixed volume, 1.0-mL w/bottle	2	each	21113-02
Flask, Erlenmeyer, 125-mL, PMP w/cap	2	each	20898-43
Pour-Thru Cell Assembly	1	each	59404-00

Required Standards

Phosphate Standard Solution, 1.00-mg/L as PO_4^{3-}	500 mL	2569-49
Phosphate Standard Solution, Voluette® ampule, 10-mL, 50-mg/L PO_4^{3-}	16/pkg	171-10
Phosphate Standard Solution, 15-mg/L PO_4^{3-}	100 mL	14243-42

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DR/2400

Phosphorus, Reactive (Orthophosphate)

Method 8178

Amino Acid Method*

(0.23 to 30.00 mg/L PO_4^{3-})

Scope and Application: For water, wastewater, and seawater

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See *Section 2.6.2 Reagent Blank* on page *20*.
- Substitute the contents of one Amino Acid Reagent Powder Pillow (Cat. No. 804-99) for 1 mL of amino acid reagent solution in *step 4*, if desired.

Method 8178

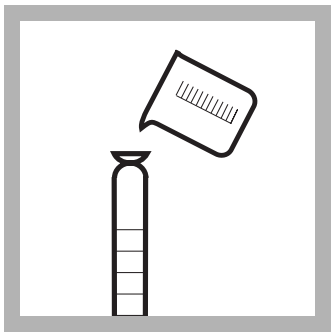
1. Touch

Hach Programs.

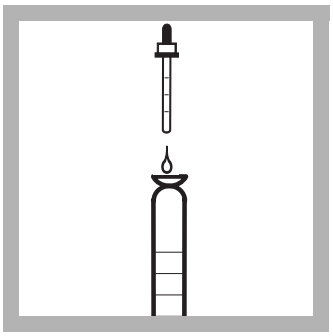
Select program

485 P React. Amino.

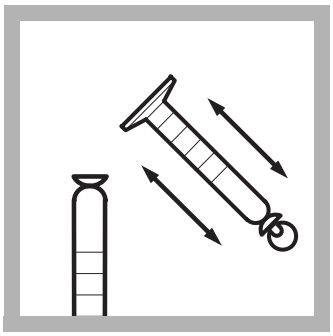
Touch **Start**.



2. Fill a 25-mL mixing cylinder with 25 mL of sample.

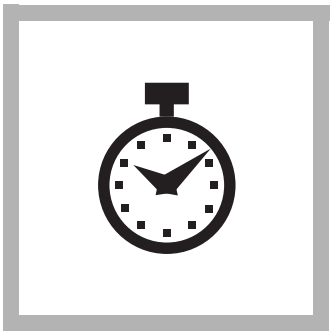


3. Add 1 mL of Molybdate Reagent using a 1-mL calibrated dropper.



4. Add 1 mL of Amino Acid Reagent Solution. Stopper and invert several times to mix (this is the prepared sample).

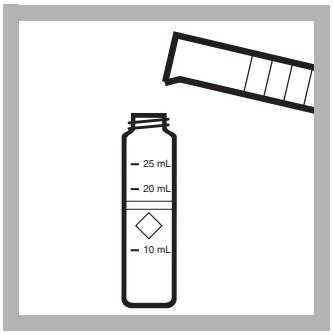
A blue color will form if phosphate is present.



5. Touch the timer icon.

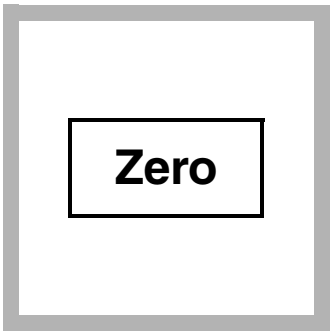
Touch **OK**.

A 10-minute reaction period will begin. Continue with *step 6* while the timer is running.



6. Pour 25 mL of sample into a round sample cell (this is the blank).

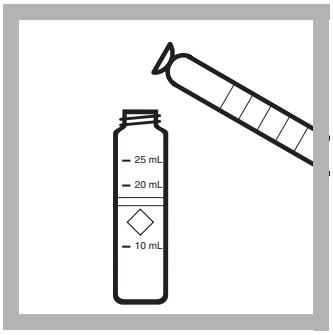
7. When the timer beeps, place the blank into the cell holder.



8. Touch **Zero**.

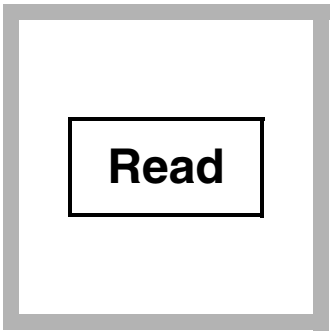
The display will show:

0.00 mg/L PO_4^{3-}



9. Pour the prepared sample into a round sample cell.

10. Place the prepared sample into the cell holder.



11. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium (Ca^{2+})	Greater than 10,000 mg/L as $CaCO_3$
Chloride	Greater than 150,000 mg/L Cl^-
Colored samples	Add 1 mL of 10 N Sulfuric Acid Standard Solution (Cat. No. 931-53) to another 25-mL sample. Use this instead of untreated sample as the blank to zero the instrument. Use a pipet and pipet filler to measure the sulfuric acid standard.
High salt levels (Na^+)	May cause low results. To eliminate this interference, dilute the sample until two successive dilutions yield about the same result.
Magnesium	Greater than 40,000 mg/L as $CaCO_3$
Nitrites (NO_2^-)	Bleach the blue color. Remove nitrite interference by adding 0.05 g of sulfamic acid (Cat. No. 2344-14) to the sample. Swirl to mix. Continue with Step 4.
Phosphates, high levels (PO_4^{3-})	As the concentration of phosphate increases, the color changes from blue to green, then to yellow and finally to brown. The brown color may suggest a concentration as high as 100,000 mg/L PO_4^{3-}. If a color other than blue is formed, dilute the sample and retest.
Sulfide (S^{2-})	Sulfide interferes. For samples with sulfide concentration less than 5 mg/L sulfide interference may be removed by oxidation with Bromine Water as follows: **1.** Measure 25 mL of sample into a sample cell. **2.** Add Bromine Water (Cat. No. 2211-20) drop-wise with constant swirling until permanent yellow color develops. **3.** Add Phenol Solution (Cat. No. 2112-20) drop-wise until the yellow color just disappears. Proceed with *step 4*.
Temperature	For best results, sample temperature should be 21 ±3 °C (70 ±5 °F).
Turbidity	May give inconsistent results for two reasons. Some suspended particles may dissolve because of the acid used in the test. Also, desorption of orthophosphate from particles may occur. For highly turbid samples, add 1 mL of 10 N Sulfuric Acid Standard Solution (Cat. No. 931-53) to another 25-mL sample. Use this instead of untreated sample as the blank to zero the instrument. Use a pipet and pipet filler to measure the sulfuric acid standard.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use a commercial phosphate-based detergent for cleaning glassware because the phosphate content will contaminate the sample.

Analyze samples immediately for best results. If prompt analysis is not possible, preserve samples by filtering immediately and store at 4 °C (39 °F) for up to 48 hours. The sample should have a neutral pH (6–8) and be at room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.

3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Section 3.2.2* for more information.
4. Snap the neck off a Phosphate 2-mL Ampule Standard, 500-mg/L PO_4^{3-}.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 10-mg/L Phosphate Standard Solution. Perform the amino acid procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 10-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision

Standard: 10.00 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
485	9.86–10.14 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.23 mg/LPO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

In a highly acidic solution, ammonium molybdate reacts with orthophosphate to form molybdophosphoric acid. This complex is then reduced by the amino acid reagent to yield an intensely colored molybdenum blue compound. Test results are measured at 530 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
High Range Reactive Phosphorus Reagent Set		100 tests	22441-00
Includes:			
Amino Acid Reagent	1 mL	100 mL MDB	1934-32
Molybdate Reagent	1 mL	100 mL MDB	2236-32

Required Apparatus

Cylinder, 25-mL, graduated, mixing	1	each	1896-40
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Phosphate Standard Solution, 10-mg/L		946 mL	14204-16
Phosphate Standard Solution, 2-mL Voluette® Ampule, 500-mg/L PO_4^{3-}		16/pkg	14242-20
Water, deionized		4 liters	272-56

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HACH COMPANY
WORLD HEADQUARTERS
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FAX: (970) 669-2932

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Phosphorus, Reactive (Orthophosphate)

Method 8114 **Molybdovanadate Method***

Reagent Solution or AccuVac® Ampuls **(0.3 to 45.0 mg/L PO_4^{3-})**

Scope and Application: For water and wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- For best results, sample temperature should be 20–25 °C (68–77 °F).
- After adding reagent, a yellow color will form if phosphate is present. The blank will be slightly yellow because of the reagent.

Reagent Solution Method 8114

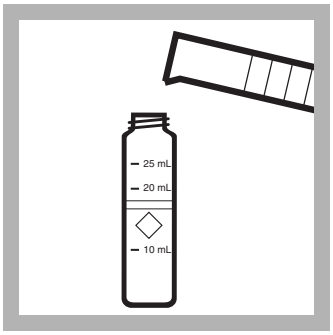
1. Touch

Hach Programs.

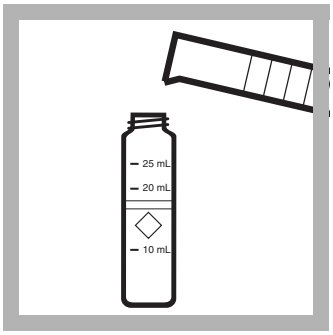
Select program

480 P React. Mo.

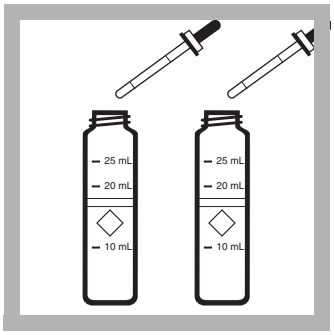
Touch **Start**.



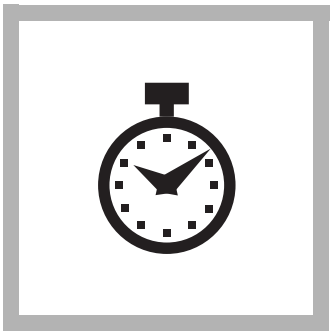
2. Using a graduated cylinder, fill a round sample cell (the blank) with 25 mL of deionized water.



3. Using a graduated cylinder, fill a round sample cell (the prepared sample) with 25 mL of sample.



4. Add 1.0 mL of Molybdovanadate Reagent to each sample cell. Swirl to mix.

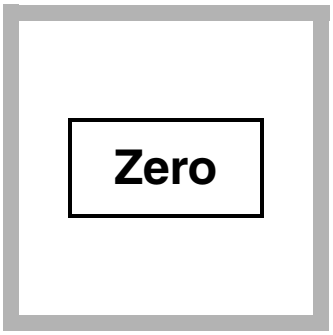


5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.0 mg/L PO_4^{3-}

8. Wipe the prepared sample and place it into the cell holder.

Touch **Read.**

Results will appear in mg/L PO_4^{3-}.

AccuVac Ampul — Method 8114

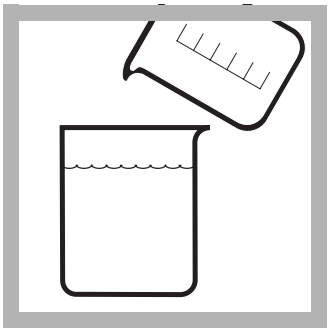
1. Touch

Hach Programs.

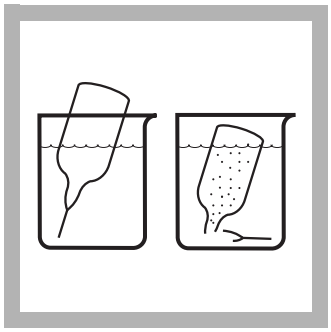
Select program

482 P React. Mo. AV.

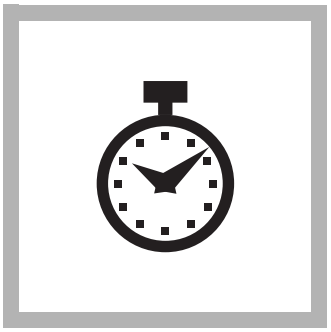
Touch **Start**.



2. Collect 40 mL of sample in one 50-mL beaker. Collect 40 mL of deionized water in another 50-mL beaker.



3. Fill a Molybdovanadate Reagent AccuVac Ampul with sample. Fill another ampule with deionized water. Keep the tips immersed while the ampules fill completely.

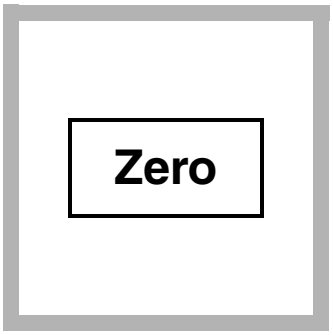


4. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.

5. When the timer beeps, place the blank into the cell holder.

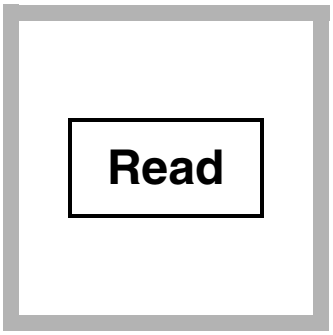


6. Touch **Zero**.

The display will show:

0.0 mg/L PO_4^{3-}

7. Place the prepared sample into the cell holder.



8. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
Arsenate	Only interferes if sample is heated.
Iron, ferrous	Blue color caused by ferrous iron does not interfere if concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Only interferes if sample is heated.
Sulfide	Causes a negative interference. **1.** Measure 50 mL of sample into an Erlenmeyer flask. **2.** Add Bromine Water (Cat. No. 2211-20) drop-wise with constant swirling until a permanent yellow color develops. **3.** Add Phenol Solution (Cat. No. 2112-20) drop-wise until the yellow color just disappears. Proceed with step *3* (step *2* if using AccuVac procedure).
pH, extreme or highly buffered samples	May exceed buffering capacity of reagents. See *Section 3.3 Interferences* on page *41*. May require pretreatment. pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause negative interference.

The following do not interfere in concentrations up to 1000 mg/L:

Table 2 Non-Interfering Substances

Pyrophosphate	Tetraborate selenate	Benzoate
Citrate	Lactate	Formate
Oxalate	Tartrate	Salicylate
Al^{3+}	Fe^{3+}	Mg^{2+}
Ca^{2+}	Ba^{2+}	Sr^{2+}
Li^{+}	Na^{+}	K^{+}
NH^{4+}	Cd^{2+}	Mn^{2+}
NO_3^{-}	NO_2^{-}	SO_4^{2-}
SO_3^{2-}	Pb^{2+}	Hg^{+}
Hg^{2+}	Sn^{2+}	Cu^{2+}
Ni^{2+}	Ag^{+}	U^{4+}
Zr^{4+}	AsO_3^{-}	Br^{-}
CO_3^{2-}	ClO_4^{-}	CN^{-}
IO_3^{-}	SiO_4^{4-}	

Sample Collection, Storage and Preservation

Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use a commercial detergent because the phosphate content will contaminate the sample.

Analyze samples as soon as possible for best results. If samples cannot be analyzed promptly, store the sample for up to 48 hours at 4 °C (39 °F) or below. Warm to room temperature before analyzing.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Phosphate 2-mL Ampule Standard, 500-mg/L PO_4^{3-}.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch

View: Fit, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 10-mg/L phosphate standard solution in place of the sample. Perform the molybdovanadate procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 10-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision
Standard: 10.0 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
480	9.8–10.2 mg/L PO_4^{3-}
482	9.7–10.3 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration Program 480	ΔConcentration Program 482
0.0 mg/L PO_4^{3-}	0.010	0.3 mg/L PO_4^{3-}	0.3 mg/L PO_4^{3-}
45.0 mg/L PO_4^{3-}	0.010	0.4 mg/L PO_4^{3-}	0.4 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

In the molybdovanadate method, orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. In the presence of vanadium, yellow molybdovanadophosphoric acid is formed. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 430 nm.

Phosphorus, Reactive (Orthophosphate)

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Molybdovanadate Reagent	2.0 mL	100 mL MDB	20760-32
or Molybdovanadate Reagent AccuVac® Ampuls	2	25/pkg	25250-25
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Beaker, 50-mL	2	each	500-41H
Cylinder, graduated, 25-mL	1	each	508-40
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Phosphate Standard Solution, 10-mg/L as PO_4^{3-}		946 mL	14204-16
Phosphate Standard Solution, 2-mL PourRite® Ampule, 500-mg/L as PO_4^{3-}		20/pkg	14242-20

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Phosphorus, Reactive (Orthophosphate)

Method 8114

Molybdovanadate Method*

Test 'N Tube™ Vials

HR (1.0 to 100.0 mg/L PO_4^{3-})

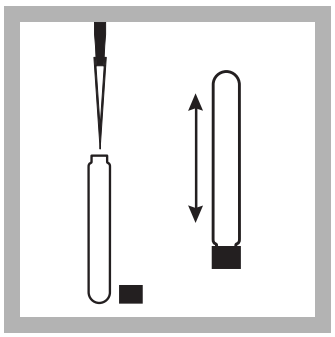
Scope and Application: For water and wastewater

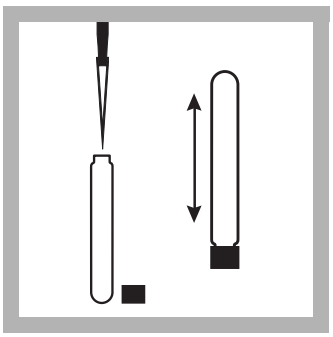
* Adapted from *Standard Methods for the Examination of Water and Wastewater*

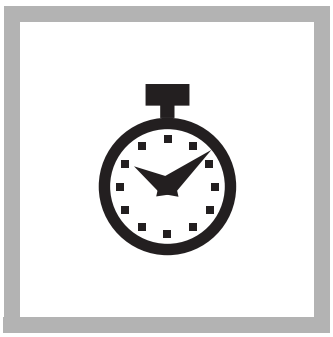
Tips and Techniques

- Reagent blanks for each lot of reagents may be used more than once. At room temperature, the reagent blank is stable for up to three weeks.
- The seven-minute reaction time in *step 4* is for samples at 23 °C. For samples at 13 °C, wait 15 minutes. For samples at 33 °C, wait two minutes.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Final samples will contain molybdenum. Also, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Consult the Material Data Safety Data Sheet for information specific to the reagents used.

Test 'N Tube — Method 8114







1. Touch

Hach Programs.

Select program

540 P React. HR TNT.

Touch **Start**.

2. Use a TenSette® Pipet to add 5.0 mL of deionized water to a Reactive High Range Phosphorus Test 'N Tube Vial (this is the blank).

Cap and invert to mix.

3. Use a TenSette Pipet to add 5.0 mL of sample to a Reactive High Range Phosphorus Test 'N Tube Vial (this is the sample).

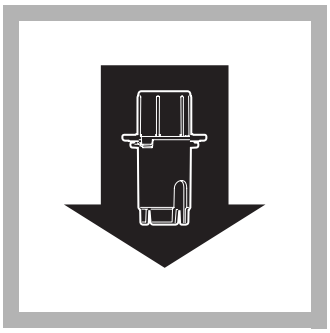
Cap and invert to mix.

4. Touch the timer icon.

Touch **OK**.

A 3-minute reaction period will begin.

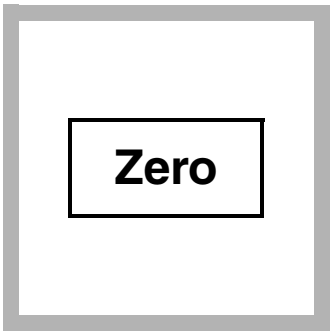
Read the sample within two minutes after the timer beeps.



5. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

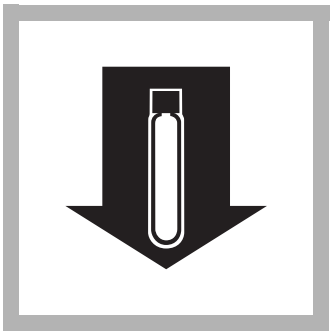
When the timer beeps, place the sample vial into the adapter.



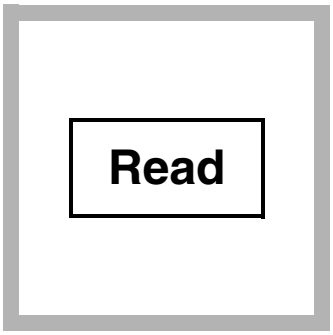
6. Touch **Zero**.

The display will show:

0.0 mg/L PO_4^{3-}



7. Place the sample vial into the cell adapter.



8. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Arsenate	Only interferes if the sample is heated.*
Iron, ferrous	Blue color caused by ferrous iron does not interfere if iron concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Only interferes if the sample is heated.*
Sulfide	Causes a negative interference. Remove interference as follows: **1.** Measure 25 mL of sample into a 50-mL beaker. **2.** Add Bromine Water (Cat. No. 2211-20) drop-wise with constant swirling until a permanent yellow color develops. **3.** Add Phenol Solution (Cat. No. 2112-20) drop-wise until the yellow color just disappears. Proceed with *step 1*.
Extreme pH or highly buffered samples	May exceed buffering capacity of the reagents. Samples may require pretreatment. Sample pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause a negative interference.
Temperature, Cold (less than 20 °C)	Causes a negative interference.
Temperature, Hot (greater than 25 °C)	Causes a positive interference.
The following do not interfere in concentrations up to 1000 mg/L: Pyrophosphate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, salicylate, Al^{3+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Li^{+}, Na^{+}, K^{+}, NH_4^{+}, Cd^{2+}, Mn^{2+}, NO_3^{-}, NO_2^{-}, SO_4^{2-}, SO_3^{2-}, Pb^{2+}, Hg^{+}, Hg^{2+}, Sn^{2+}, Cu^{2+}, Ni^{2+}, Ag^{+}, U^{4+}, Zn^{4+}, AsO_3^{-}, Br^{-}, CO_3^{2-}, ClO_4^{-}, CN^{-}, IO_3^{-}, SiO_4^{4-}.	

* Gentle warming of the sample to room temperature will not prevent this substance from interfering.

Sampling and Storage

Collect samples in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

For best results, analyze the samples immediately after collection. If prompt analysis is impossible, preserve the samples for up to 48 hours by filtering immediately and storing at 4 °C. The sample should be at room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Clean glassware with 1:1 hydrochloric acid solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Snap the neck off a 10-mL Voluette® Ampule of Phosphate Standard Solution, 500-mg/L PO_4^{3-} (Cat. No. 14242-10).
6. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 10 mL of sample. Use the TenSette Pipet to add 0.1, 0.2 mL, and 0.3 mL of standard, respectively to each sample and mix thoroughly.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32*, for more information.

Standard Solution Method

1. Use a 50-mg/L PO_4^{3-} standard in place of the sample. Perform this procedure as described.
2. To adjust the calibration curve using the reading obtained with the 50-mg/L PO_4^{3-} phosphate standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 50.0 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
540	48.7–51.3 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	1.0 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. In the presence of vanadium, yellow molybdovanadophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 420 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
High Range Reactive Phosphorus Test 'N Tube™ Reagent Set		50 vials	27673-45
Includes:			
(1) Reactive High Range Phosphorus Test 'N Tube Vials	1	50/pkg	*
(2) Water, deionized	5 mL	100 mL	272-42

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Dropper, LDPE, 0.5–1.0 mL	1	20/pkg	21247-20
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	1	50/pkg	21997-96
Test Tube Rack	1–3	each	18641-00

Required Standards

Phosphate Standard Solution, 50-mg/L, as PO_4^{3-}		500 mL	171-49
Phosphate Standard Solution, Voluette® ampule, 500-mg/L as PO_4^{3-}, 10-mL		16/pkg	14242-10

* Not available separately.

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 8114 **Molybdovanadate Rapid Liquid Method***

Pour-Thru Cell **HR (0.3 to 45.0 mg/L PO_4^{3-})**

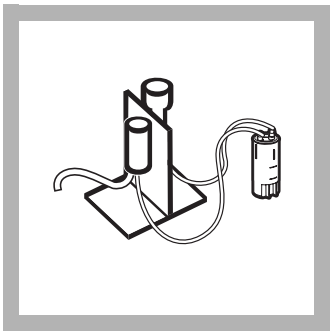
Scope and Application: For treated and natural waters

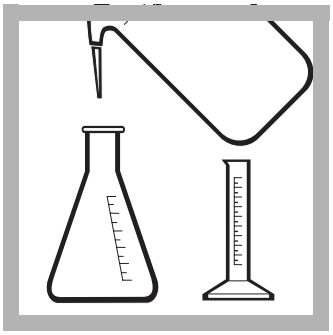
* Adapted from Standard Methods for the Examination of Water and Wastewater.

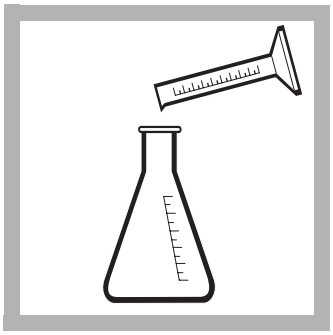
Tips and Techniques

- Clean the Pour-Thru cell and all labware as specified in *Treating Analysis Labware on page 4*.
- Protect the Pour-Thru cell from contamination when not in use by inverting a small beaker over the top of the glass funnel.
- Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Consult the Material Data Safety Data Sheet for information specific to the reagents used.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

DR/2400 Pour-Thru Cell Method 8114







1. Touch

Hach Programs.

Select program

489 P React. Mo. HR RL.

Touch **Start**.

2. Install the Pour-Thru Cell and multi-pathlength cell adapter in the sample cell compartment.

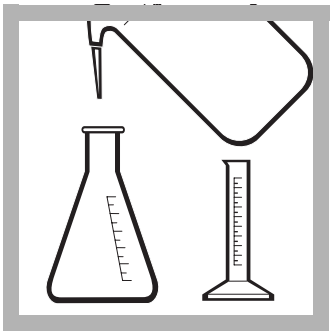
Note: *See Section 2.6.1 on page 26 in the Instrument manual for installation details.*

Flush with 50-mL of deionized water.

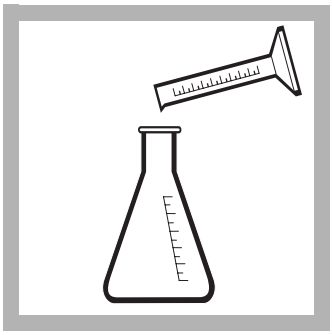
Note: *Use the 25-mm cell pathlength.*

3. Rinse a clean plastic 125-mL Erlenmeyer flask and a 25-mL graduated cylinder with deionized water.

4. Measure 25 mL of deionized water in the graduated cylinder. Pour the water into the flask (this is the blank).



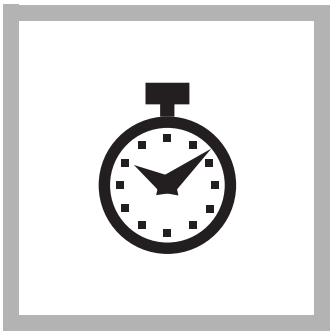
5. Rinse another clean plastic 125-mL Erlenmeyer flask and a 25-mL graduated cylinder with deionized water.



6. Measure 25 mL of sample in the graduated cylinder. Pour the water into the flask (this is the sample).

7. Add 1.0 mL of Molybdovanadate Reagent to each flask using a Repipet Jr. Dispenser. Swirl to mix.

A yellow color will develop in the sample if phosphate is present. A small amount of yellow may be present in the blank due to the reagent.

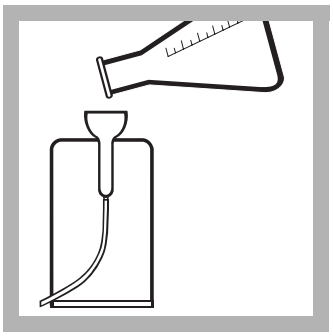


8. Touch the timer icon.

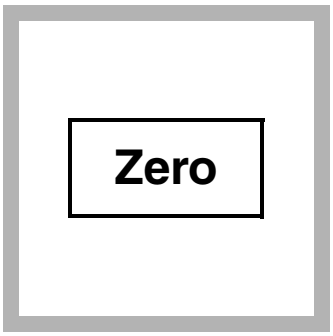
Touch **OK**.

A three-minute reaction period will begin.

If the sample concentration is greater than 24 mg/L PO_4^{3-}, read at exactly three minutes or make a 1:1 dilution of the sample and begin the test again.



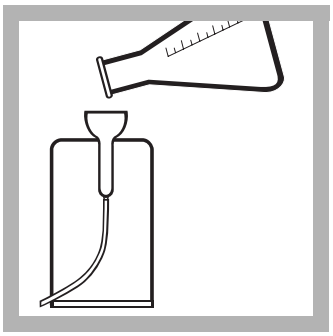
9. When the timer beeps, pour the blank from the flask into the Pour-Thru Cell.



10. After the flow stops, touch **Zero**.

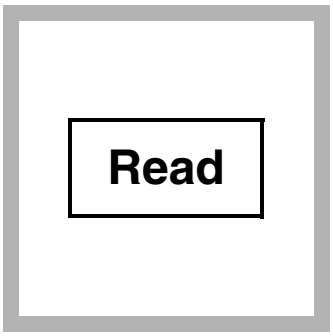
The display will show:

0.0 mg/L PO_4^{3-}



11. Pour the prepared sample from the flask into the Pour-Thru Cell.

Flush the Pour-Thru Cell with 50 mL of deionized water.



12. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Arsenate	Negative interference. Positive interference if sample is heated.
Bismuth	Negative interference.
Fluoride	Negative interference.
Iron, Ferrous	Blue color is caused by ferrous iron but this does not affect results if the ferrous iron concentration is less than 100 mg/L.
Molybdate	Negative interference.
Silica	Positive interference if sample is heated.
Sulfide	Negative interference. Sulfide interference may be removed by oxidation with Bromine Water as follows: **1.** Measure 25 mL of sample into a flask. **2.** Add Bromine Water (Cat. No. 2211-20) drop-wise with constant swirling until permanent yellow color develops. **3.** Add Phenol Solution (Cat. No. 2112-20) drop-wise until the yellow color just disappears. Proceed with step 7.
Thiocyanate	Negative interference.
Thiosulfate	Negative interference.
Thorium	Negative interference.
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment; see *Section 3.3 Interferences* on page *41*.

The following do not interfere in concentrations up to 1000 mg/L:

Pyrophosphate	Tetraborate selenate	Benzoate
Citrate	Lactate	Formate
Oxalate	Tartrate	Salicylate
Al^{3+}	Fe^{3+}	Mg^{2+}
Ca^{2+}	Ba^{2+}	Sr^{2+}
Li^{+}	Na^{+}	K^{+}
NH^{4+}	Cd^{2+}	Mn^{2+}
NO_3^{-}	NO_2^{-}	SO_4^{2-}
SO_3^{2-}	Pb^{2+}	Hg^{+}
Hg^{2+}	Sn^{2+}	Cu^{2+}
Ni^{2+}	Ag^{+}	U
Zr^{4+}	AsO_3^{-}	Br^{-}
CO_3^{2-}	ClO_4^{-}	CN^{-}
IO_3^{-}	SiO_4^{4-}	

Sampling and Storage

Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use detergents that contain phosphate for cleaning labware.

Analyze samples immediately for best results. If prompt analysis is not possible, preserve samples by filtering immediately and storing the sample at 4 °C (39 °F) or below for up to 48 hours. Warm to room temperature before analyzing.

Treating Analysis Labware

Clean containers by normal means (do not use detergents containing phosphorus), then rinse with deionized water. Soak for several minutes in a 1:25 dilution of Molybdovanadate Reagent in deionized water. Rinse well with deionized water. Dedicate these containers for HR PO_4^{3-} analysis. Fill the Pour-Thru Cell with this same mixture of Molybdovanadate reagent and deionized water, and let stand for several minutes. Rinse with 50 mL of deionized water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. Remove the color by rinsing with a 1:5 dilution of ammonium hydroxide (Cat. No. 106-49), followed by several deionized water rinses. Invert a beaker over the glass funnel of the Pour-Thru Cell when not in use.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Phosphate Voluette® Ampule Standard Solution, 500-mg/L as PO_4^{3-}.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery. Each 0.1-mL addition of standard should cause an increase of 2.0 mg/L PO_4^{3-} or 0.65 mg/L P.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 10.0-mg/L Phosphate Standard in place of the sample. Results should be between 9.0 and 11.0 mg/L phosphate.
2. To adjust the calibration curve using the reading obtained with the 10-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 10.0 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
489	9.9–10.1 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
0.0 ppm PO_4^{3-}	0.010	0.3 mg/L PO_4^{3-}
45.0 ppm PO_4^{3-}	0.010	0.4 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

In the molybdovanadate method, orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 430 nm.

Phosphorus, Reactive

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Molybdovanadate Reagent	2	500 mL	20760-49
Water, deionized	25	4 L	272-56

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cylinder, graduated, 25-mL, poly	1	each	1081-40
Dispenser, fixed volume, 1.0-mL, w/bottle	1	each	21113-02
Flask, Erlenmeyer, 125-mL, PMP w/cap	2	each	20898-43
Pour-Thru Cell Kit	1	each	59404-00

Required Standards

Phosphate Standard Solution, 10-mg/L as PO_4^{3-}		946 mL	14204-16
Phosphate Standard Solution, Voluette® ampule, 10-mL, 500-mg/L as PO_4^{3-}		16/pkg	14242-10

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DR/2400

Phosphorus, Reactive (Orthophosphate)

★**Method 8048** **PhosVer 3 (Ascorbic Acid) Method***

Powder Pillows or AccuVac® Ampuls **(0.02 to 2.50 mg/L PO_4^{3-})**

Scope and Application: For water, wastewater, and seawater; USEPA Accepted for reporting for wastewater analyses**

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** Procedure is equivalent to USEPA method 365.2 and Standard Method 4500-P-E for wastewater.

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See *Section 2.6.2 Reagent Blank* on page *20* for more information.
- A blue color will develop if phosphorus is present.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows — Method 8048

1. Touch

Hach Programs.

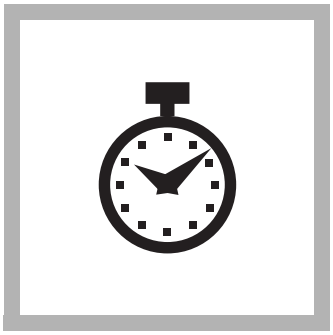
Select program

490 P React. PV.

Touch **Start**.

2. Fill a sample cell with 10-mL of sample.

3. Add the contents of one PhosVer 3 phosphate Powder Pillow to the cell. Immediately cap and invert to mix (this is the prepared sample).



4. Touch the timer icon.

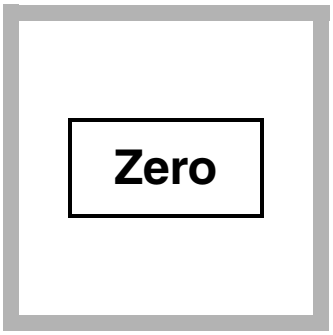
Touch **OK**.

A two-minute reaction period will begin.

If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.

5. Fill another sample cell with 10 mL of sample (this is the blank).

6. When the timer beeps, wipe the blank and place it into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L PO_4^{3-}

8. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

AccuVac® Ampul — Method 8048

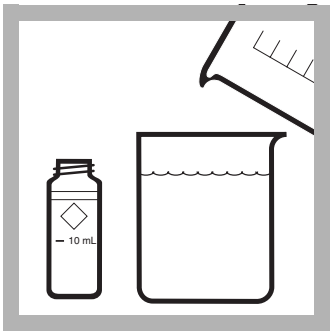
1. Touch

Hach Programs.

Select program

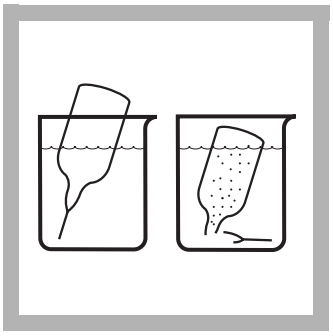
492 P React. PV AV.

Touch **Start**.

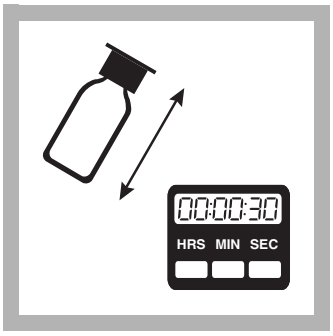


2. Fill a sample cell with 10-mL of sample. (This is the blank.)

Note: *Collect at least 40 mL of sample in a 50-mL beaker.*

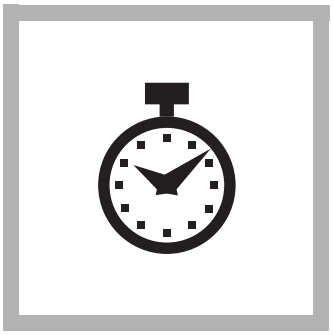


3. Fill a PhosVer 3 Phosphate AccuVac Ampul with sample. Keep the tip immersed while the ampule fills completely.



4. Place an ampule cap securely over the tip of the ampule. Shake the ampule for approximately 30 seconds.

Note: *Accuracy is unaffected by undissolved powder.*



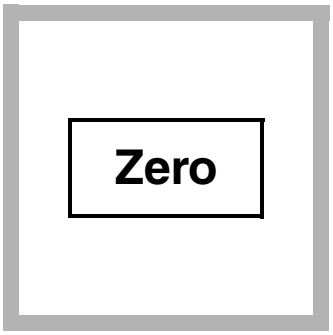
5. Touch the timer icon.

Touch **OK**.

A two-minute reaction period will begin.

If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.

6. When the timer beeps, wipe the blank and place it into the cell holder.



7. Touch **Zero**.

The display will show:

0.00 mg/L PO_4^{3-}

8. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level.
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Hydrogen Sulfide	Interferes at any level
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. pH 2–10 is recommended.
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Turbidity (large amounts) or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. For highly turbid or colored samples, add the contents of one Phosphate Pretreatment Powder Pillow (Cat. No. 14501-99) to 25 mL of sample. Mix well. Use this solution to zero the instrument.
Zinc	Greater than 80 mg/L

Sample Collection, Storage, and Preservation

Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.

Analyze samples immediately for best results. If prompt analysis is not possible, preserve samples by filtering immediately and storing at 4 °C (39 °F) for up to 48 hours. The sample should be at room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a Phosphate 10-mL Ampule Standard, 50-mg/L PO_4^{3-}.
5. Prepare a 0.1-mL sample spike by adding 0.1 mL of standard to the unspiked sample. Touch the timer icon. After the timer beeps, read the result.
6. Prepare a 0.2-mL sample spike by adding 0.1 mL of standard to the 0.1-mL sample spike. Touch the timer icon. After the timer beeps, read the result.
7. Prepare a 0.3-mL sample spike by adding 0.1 mL of standard to the 0.2-mL sample spike. Touch the timer icon. After the timer beeps, read the result. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac ampules, fill three Mixing Cylinders (Cat. No. 1896-41) with 50 mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL Beakers (Cat. No. 500-41H). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 1.0-mg/L phosphate standard solution in place of the sample. Perform the procedure as describe above.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision

Standard: 1.00 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
490	0.97–1.03 mg/L PO_4^{3-}
492	0.98–1.02 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	Program	ΔConcentration
Entire range	0.010	490	0.02 mg/L PO_4^{3-}
Entire range	0.010	492	0.02 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
PhosVer™ 3 Phosphate Reagent Powder Pillows, 10-mL	1 pillow	100/pkg	21060-69
or PhosVer™ 3 Phosphate Reagent AccuVac® Ampuls	1 ampule	25/pkg	25080-25

Required Apparatus

Beaker, 50-mL	1	each	500-41H
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Stopper for 18-mm tube	1	25/pkg	1731-25

Required Standards

Phosphate Standard Solution, 10-mL Voluette® Ampul, 50-mg/L as PO_4	16/pkg	171-10
Phosphate Standard Solution, 50-mg/L as PO_4	500 mL	171-49
Phosphate Standard Solution, 1-mg/L as PO_4	500 mL	2569-49
Water, deionized	4 liters	272-56

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3/04 1ed

DR/2400 Phosphorus, Reactive (Orthophosphate)

★**Method 8048** **PhosVer™ 3 Method**

Test 'N Tube™ Vials **(0.06 to 5.00 mg/L PO_4^{3-} or 0.02 to 1.60 mg/L P)**

Scope and Application: For water, wastewater, and seawater; USEPA accepted for reporting wastewater analysis*

* Procedure is equivalent to USEPA Method 365.2 and Standard Method 4500-P E for wastewater.

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See *Section 2.6.2 Reagent Blank* on page *20* for more information.

Test 'N Tube Method 8048

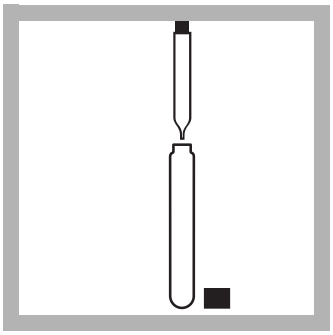
1. Touch

Hach Programs.

Select program

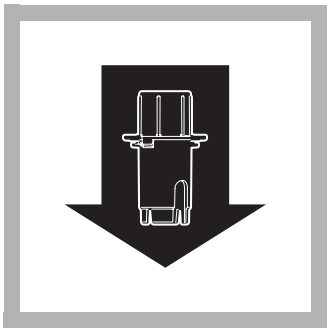
535 P React. PV TNT.

Touch **Start**.



2. Use a TenSette® Pipet to add 5.0 mL of sample to a Reactive Phosphorus Test 'N Tube Dilution Vial. Cap and mix.

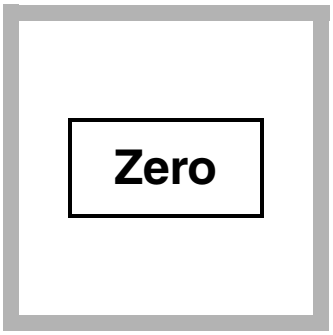
3. Wipe the outside of the vial with a damp towel, followed by a dry one, to remove fingerprints or other marks.



4. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

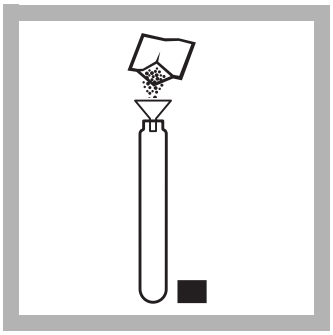
Place the vial into the adapter.



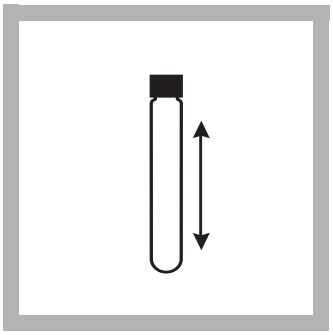
5. Touch **Zero**.

The display will show:

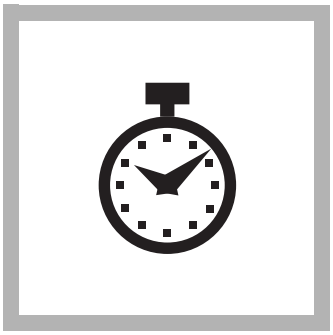
0.00 mg/L PO_4^{3-}



6. Using a funnel, add the contents of one PhosVer 3 Phosphate Powder Pillow to the vial.



7. Cap the vial tightly and shake for 10–15 seconds. The powder will not dissolve completely.



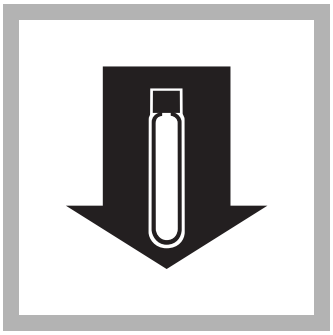
8. Touch the timer icon

Touch **OK**.

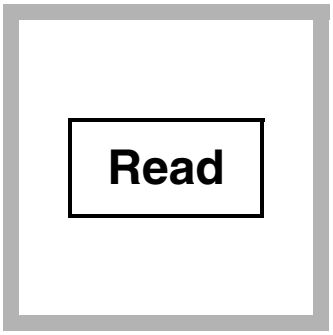
A two-minute reaction period will begin.

Read samples between two and eight minutes after adding the PhosVer 3 reagent.

9. Wipe the outside of the vial with a damp towel, followed by a dry one, to remove fingerprints or other marks.



10. When the timer beeps, place the vial into the cell adapter.



11. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 200 mg/L
Arsenate	All levels
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
Silica	Greater than 50 mg/L

(continued)

Interfering Substance	Interference Levels and Treatments (continued)
Silicate	Greater than 10 mg/L
Sulfide	Greater than 6 mg/L. Remove sulfide interference as follows: **1.** Measure 25 mL of sample into a 50-mL beaker. **2.** Swirling constantly, add Bromine Water (Cat. No. 2211-20) drop-wise until a permanent yellow color appears. **3.** Swirling constantly, add Phenol Solution (Cat. No. 2112-20) drop-wise just until the yellow color disappears. Proceed with *step 1* of the phosphorus procedure.
Turbidity	Large amounts may cause inconsistent results in the test because the acid present in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment.

Sample Collection, Storage, and Preservation

Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples up to 48 hours by filtering immediately and storing at 4 °C. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Snap the neck off a Phosphate 2-mL Ampule Standard, 50-mg/L as PO_4^{3-}.
6. Prepare three sample spikes. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 1.0-mg/L phosphate standard solution in place of the sample. Perform the procedure as describe above.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision
Standard: 3.00 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
535	2.89–3.11 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.06 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Reactive Phosphorus Test 'N Tube™ Reagent Set (50 tests)			27425-45
Includes:			
PhosVer™ 3 Phosphate Reagent Powder Pillows	1	50/pkg	21060-46
Reactive Phosphorus Test 'N Tube Dilution Vials	1	50/pkg	*

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
Funnel, micro	1	each	25843-35
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	1	50/pkg	21997-96
Test Tube Rack	1–3	each	18641-00

Required Standards

Phosphate Standard Solution, Voluette™ Ampule, 50-mg/L as PO_4^{3-}, 2-mL	20/pkg	17120 H
Phosphate Standard Solution, 50-mg/L	500 mL	171-49
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}	500 mL	2569-49

* Not available separately

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3/04 1ed

DR/2400

Phosphorus, Reactive (Orthophosphate)

Ascorbic Acid Method

(1.5 to 15.0 mg/L PO_4^{3-})

UniCell™ Vials

Scope and Application: For water, wastewater, boiler water, surface water, and process control

Tips and Techniques

- Adjust the pH of preserved samples between pH 2–10 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results (see *Accuracy Check on page 3*).
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure the temperature of the water sample and the sample vial is between 18–20 °C. Temperatures outside of this range will affect the color of the reaction and may give inaccurate results.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

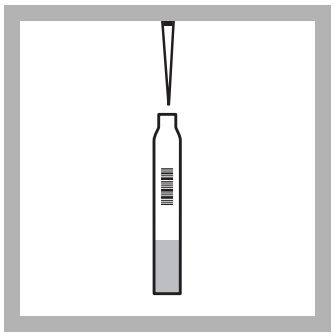
1. Touch

Hach Programs.

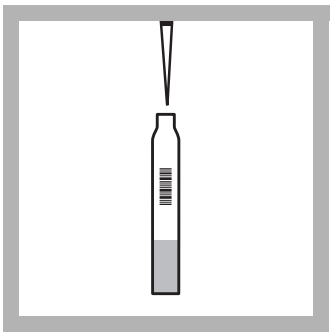
Select program

806 Phosphate, HCT 121.

Touch **Start**.

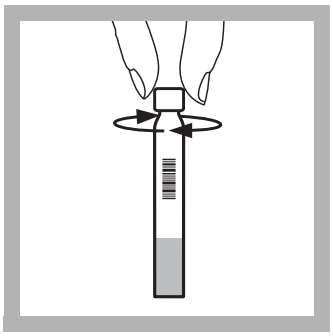


2. Pipet 1.0 mL of sample into a sample vial.

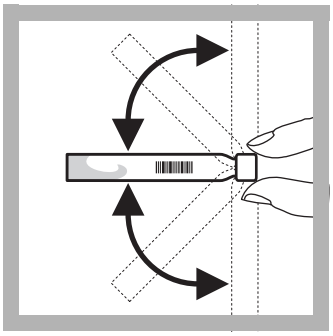


3. Pipet 0.4 mL of Reagent B (HCT 121/122 B) into the sample vial.

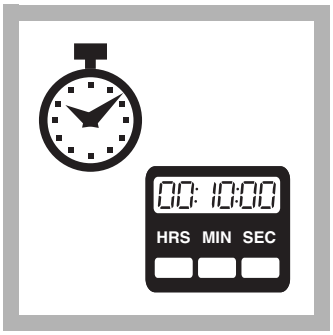
Close the Reagent B bottle immediately after use.



4. Screw a **light green** UniCap C (HCT 121 C) onto the sample vial.



5. Invert the sample vial repeatedly until the solid reagent in the cap dissolves.

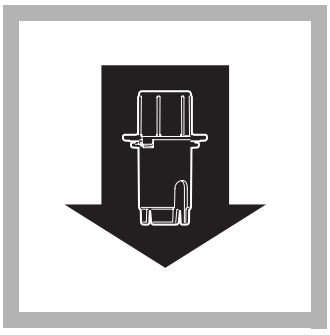


6. Touch the timer icon

Touch **OK**.

A 10-minute reaction period will begin.

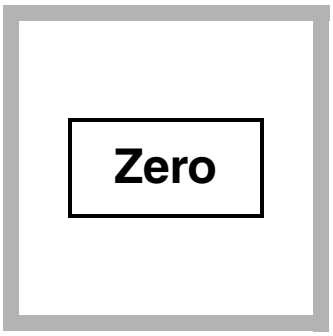
7. Wipe the outside of the zero (white cap) and sample vials with a damp towel followed by a dry one.



8. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

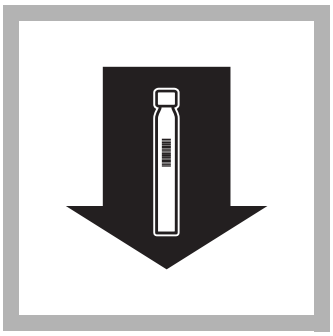
Place the zero vial into the cell adapter.



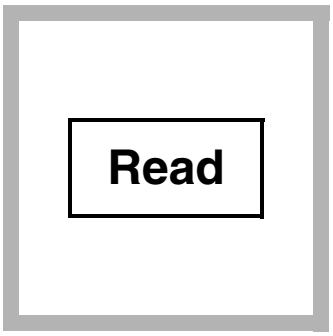
9. Touch **Zero**.

The display will show:

0.0 mg/L PO_4^{3-}
Underrange



10. When the timer beeps, place the sample vial into the cell adapter.



11. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	No interference up to:
SO_4^{2-}	20 g/L
Cl^-	10 g/L
K^+, Na^+	4 g/L
Ca^{2+}	1 g/L
Mg^{2+}	400 mg/L
Co^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, NO_2^-, Cd^{2+}, NH_4^+, Mn^{2+}, Al^{3+}, CO_3^{2-}	200 mg/L
I^-	100 mg/L

(continued)

Interfering Substance	No interference up to:
SiO_2	50 mg/L
Hg^{2+}	40 mg/L
Sn^{2+}	25 mg/L
Pb^{2+}	20 mg/L
Ag^{+}, Cr^{3+}	10 mg/L
Cr^{6+}	1 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Store in a cool dry place in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

If prompt analysis is impossible, preserve samples up to 48 hours by filtering immediately and storing at 4 °C (39 °F). Do not use mercury compounds as preservatives. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row.
5. Snap the neck off a 10-mL Voluette® Ampule of Phosphate Standard, 500 mg/L as PO_4^{3-}.
6. Prepare three sample spikes. Fill three 100-mL mixing cylinders with 100 mL of sample. Use a pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of standard, respectively to each cylinder and mix thoroughly.
7. Analyze each sample spike as described in the procedure above, starting with the 0.2-mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use the 10-mg/L Phosphate Standard Solution listed under *Optional Standards*. Perform the phosphate procedure as described.
2. To adjust the calibration curve using the reading obtained with the 10 mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 7.5 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
806	7.2–7.8 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
806	Entire range	0.010	0.2 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Phosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex. Ascorbic acid reduces this complex to phosphomolybdenum blue. Test results are measured at 880 nm.

Required Reagents

Description	Unit	Cat. No.
Phosphate - PO_4^{3-}, LR, UniCell™ HCT 121	23/pkg	HCT 121

Required Apapratus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Cylinder, mixing, 100-mL graduated	each	508-42
Digital Reactor Block 100	each	DRB 100
Pipettor, Jencons, 100–1000 µL	each	27949-00
Tips, replacement for 27949-00	400/pkg	27950-00
Rack, Cooling, Test Tube	each	18641-00

Optional Standards

Hydrochloric Acid Solution, 1:1	500 mL	884-49
Phosphate Standard, 10-mL Voluette® Ampule, 500-mg/L as PO_4	16/pkg	14242-10
Phosphate Standard, 10-mg/L as PO_4	946 mL	14204-16

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DR/2400

Phosphorus, Reactive (Orthophosphate)

Ascorbic Acid Method

(6.0 to 60.0 mg/L PO_4^{3-})

UniCell™ Vials

Scope and Application: For water, wastewater, boiler water, surface water, and process control

Tips and Techniques

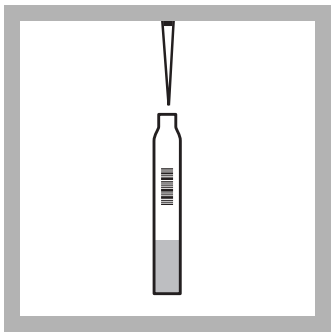
- Adjust the pH of preserved samples to between pH 2–10 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results (see *Accuracy Check on page 3*).
- See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is between 18–20 °C. Temperatures outside of this range will affect the color of the reaction and may give inaccurate results.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

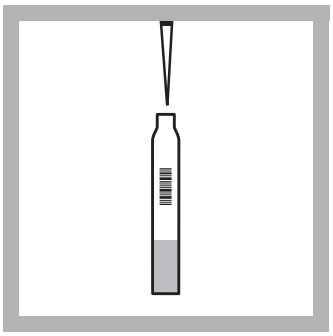
1. Touch **Hach Programs**.

Select program **804 Phosphate, HCT 122**.

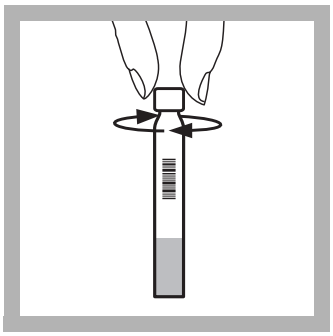
Touch **Start**.



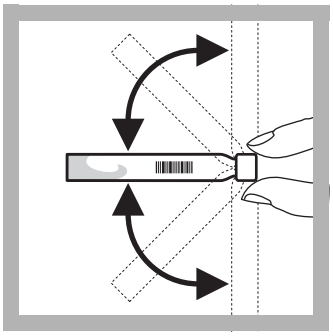
2. Pipet 0.4 mL of sample into a sample vial.



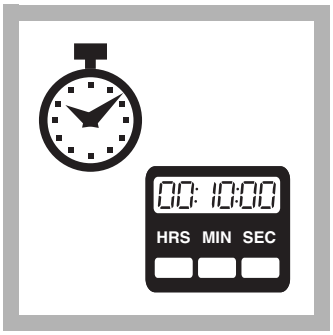
3. Pipet 0.5 mL of Reagent B (HCT 121/122 B) into the sample vial.



4. Screw a **grey** UniCap C (HCT 122 C) onto the sample vial.



5. Invert the sample vial repeatedly until the solid reagent in the cap is dissolved.

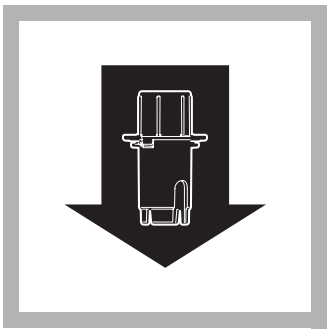


6. Touch the timer icon

Touch **OK**.

A 10-minute reaction period will begin.

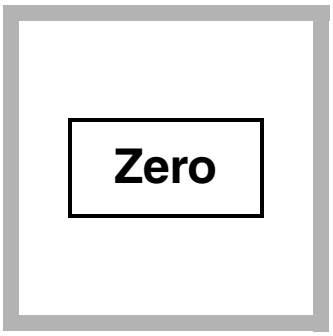
7. Wipe the outside of the zero (white cap) and sample vials with a damp towel followed by a dry one to remove fingerprints or other marks.



8. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Place the zero vial into the cell adapter.

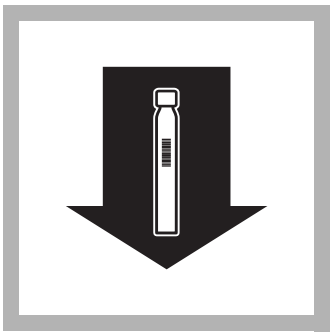


9. Touch **Zero**.

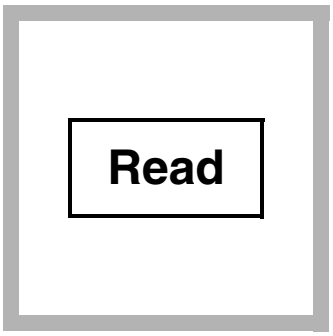
The display will show:

0.0 mg/L PO_4^{3-}

Underrange



10. When the timer beeps, place the sample vial into the cell adapter.



11. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	No interference up to:
SO_4^{2-}	5000 mg/L
Cl^-	2000 mg/L
K^+, Na^+, Ca^{2+}	1000 mg/L
Mg^{2+}, NO_3^-	500 mg/L
Co^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Cr^{3+}, I^-, NO_2^-, Cd^{2+}, Sn^{2+}, NH_4^+, Mn^{2+}, Al^{3+}, Hg^{2+}, Pb^{2+}, SiO_2	50 mg/L
Ag^+	25 mg/L
Cr^{6+}	5 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Store in a cool dry place in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

If prompt analysis is impossible, preserve samples up to 48 hours by filtering immediately and storing at 4 °C. Do not use mercury compounds as preservatives. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Clean glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Snap the neck off a Phosphate 10-mL Ampule Standard, 500-mg/L as PO_4^{3-}.
6. Prepare three sample spikes. Fill three 25-mL mixing cylinders with 25 mL of sample. Use a pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of 500-mg/L PO_4^{3-} standard, respectively, to each sample and mix thoroughly.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 30-mg/L Phosphate Standard Solution listed under *Optional Standards*. Perform the phosphate procedure as described.
2. To adjust the calibration curve using the reading obtained with the 30-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 30.0 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
804	29.5–30.5 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
804	Entire range	0.010	0.5 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Phosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex. Ascorbic acid reduces this complex to phosphomolybdenum blue. Test results are measured at 880 nm.

Required Reagents

Description	Unit	Cat. No.
Phosphate - PO_4^{3-}, HR, UniCell™ HCT 122	23/pkg	HCT 122

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Cylinder, mixing, 25-mL graduated	each	20886-40
Digital Reactor Block 100	each	DRB 100
Pipettor, Jencons, 100–1000 µL	each	27949-00
Rack, Cooling, Test Tube	each	18641-00
Tips, replacement for 27949-00	400/pkg	27950-00

Optional Standards

Hydrochloric Acid Solution, 1:1	500 mL	884-49
Phosphate Standard, 10-mL Voluette® Ampule, 500-mg/L as PO_4	100 mL	14242-10
Phosphate Standard, 30-mg/L as PO_4	946 mL	14367-16

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Phosphorus, Total, Digestion

★Method 8190 — Acid Persulfate Digestion Method*

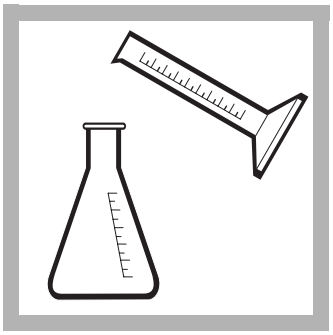
Scope and Application: For water, wastewater, and seawater; USEPA Accepted for wastewater analyses

* Adapted from *Standard Methods for the Examination of Water and Wastewater* 4500-P B & E

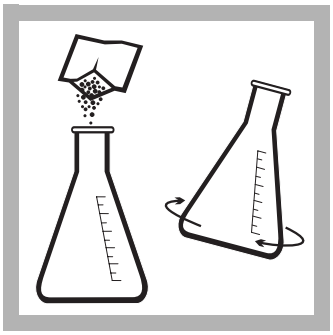
Tips and Techniques

- Rinse all glassware with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49). Rinse again with deionized water.
- The results of the reactive phosphorus test after the digestion will include the organic phosphate plus the orthophosphate and the acid-hydrolyzable (condensed) phosphate. The organic phosphate concentration is determined by subtracting results of an acid hydrolyzable phosphorus test from this result. Make sure that both results are in the same units, either mg/L PO_4^{3-} or mg/L P before subtracting.

Powder Pillows — Method 8190

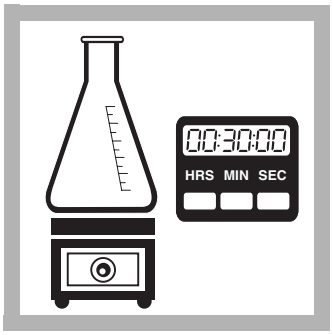


1. Use a graduated cylinder to measure 25 mL of sample. Pour the sample into a 125-mL Erlenmeyer flask.



2. Add the contents of one Potassium Persulfate Powder Pillow. Swirl to mix.

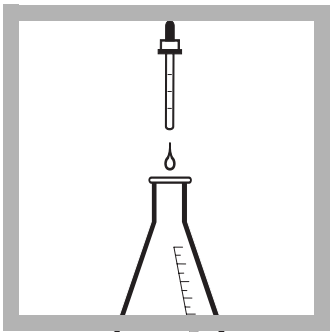
3. Use a 1-mL calibrated dropper to add 2.0 mL of 5.25 N Sulfuric Acid Solution to the flask.



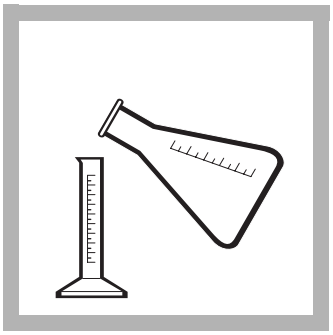
4. Place the flask on a hot plate. Boil gently for 30 minutes.
Do not boil dry.

Concentrate the sample to less than 20 mL for best recovery. After concentration, maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.

5. Cool the sample to room temperature.



6. Use a 1-mL calibrated dropper to add 2.0 mL of 5.0 N Sodium Hydroxide Solution to the flask. Swirl to mix.



7. Pour the sample into a 25-mL graduated cylinder. Adjust the volume to 25 mL with deionized water rinsings from the flask.

8. Proceed with a reactive phosphorus test of the expected total phosphorus concentration range. Extend the color development time to 10 minutes for the Ascorbic Acid method.

Interferences

Interfering Substance	Interference Levels and Treatments
Alkaline or highly buffered samples	It may be necessary to add additional acid in step 3 to drop the pH of the solution below 1.
Turbidity	Use 50 mL of sample and double the reagent quantities. Use 25 mL of the reacted sample to zero the instrument in the reactive phosphorus procedure. This compensates for any color or turbidity destroyed by this procedure.

Sample Collection, Storage, and Preservation

Analyze the samples immediately for the most reliable results. If prompt analysis is not possible, samples may be preserved up to 28 days by adjusting the pH to 2 or less with concentrated Sulfuric Acid (about 2 mL per liter) (Cat. No. 979-49) and storing at 4 °C. Warm the sample to room temperature and neutralize with 5.0 N Sodium Hydroxide (Cat. No. 2450-53) before analysis. Correct for volume additions: see *Section 3.1.3 Correcting for Volume Additions* on page 29.

Summary of Method

Phosphates present in organic and condensed inorganic forms (meta-, pyro-, or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphate by heating with acid and persulfate. Organically bound phosphates are thus determined indirectly by subtracting the result of an acid hydrolyzable phosphorus test from the total phosphorus result.

This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determining the phosphorus content of the sample. If the ascorbic acid (PhosVer 3) method is used to measure the reactive phosphorus, this method is USEPA accepted for NPDES reporting.

The following reagents and apparatus are required in addition to those required for the reactive phosphorus test.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Potassium Persulfate Powder Pillows	1 pillow	100/pkg	2451-99
Sodium Hydroxide Solution, 5.0 N	2 mL	100 mL MDB	2450-32
Sulfuric Acid Solution, 5.25 N	2 mL	100 mL MDB	2449-32
Water, deionized		4 liters	272-56

Required Apparatus

Cylinder, graduated, 25-mL	1	each	508-40
Flask, Erlenmeyer, 125-mL	1	each	505-43
Hot Plate, 4-inch diameter, 120 VAC	1	each	12067-01
Hot Plate, 4-inch diameter, 240 VAC	1	each	12067-02

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3/04 1ed

Phosphorus, Total

★Method 8190 **PhosVer™ 3 with Acid Persulfate Digestion Method**

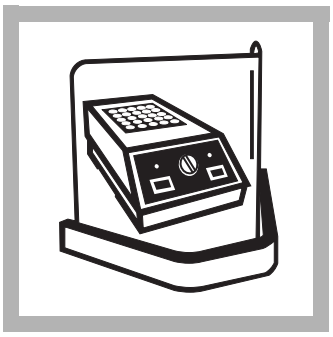
Test 'N Tube™ Vials **(0.06 to 3.50 mg/L PO_4^{3-} or 0.02 to 1.10 mg/L P)**

Scope and Application: For water, wastewater, and seawater; USEPA Accepted for reporting wastewater analyses

Tips and Techniques

- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- The test range for total phosphate is limited to 0.06 to 3.5 mg/L PO_4^{3-}. Values greater than 3.5 mg/L may be used to estimate dilution ratios, but should NOT be used for reporting purposes. If the value is greater than 3.5 mg/L, dilute the sample and repeat the digestion and the colorimetric test.
- Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA.

Test 'N Tube — Method 8190

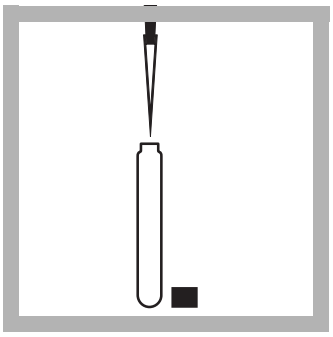


1. Turn on the COD reactor. Heat to 150 °C. Place the safety shield in front of the reactor.

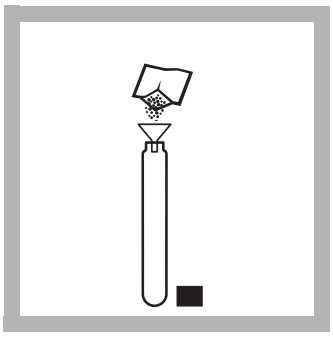
2. Touch **Hach Programs**.

Select program **536 P Total/AH PV TNT**.

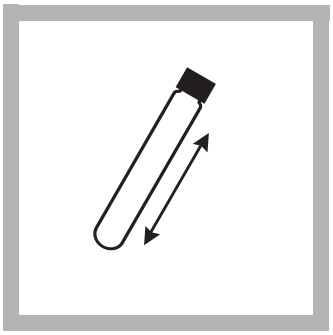
Touch **Start**.



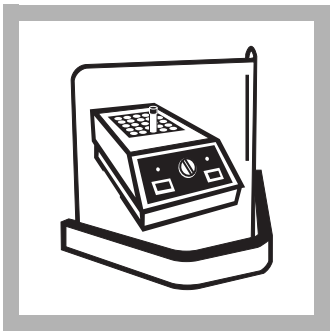
3. Use a TenSette® Pipet to add 5.0 mL of sample to a Total and Acid Hydrolyzable Test Vial.



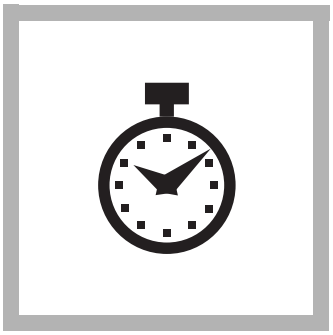
4. Use a funnel to add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.



5. Cap tightly and shake to dissolve.



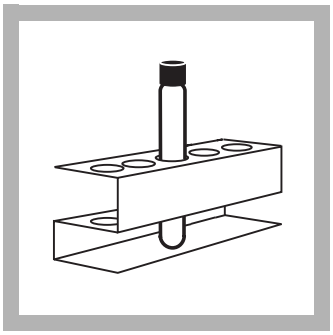
6. Place the vial into the COD Reactor.



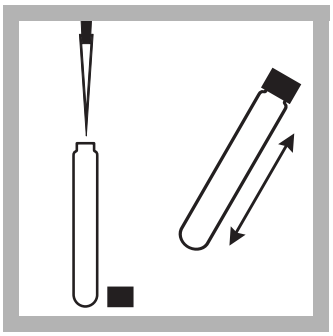
7. Touch the timer icon.

Touch **OK**.

A 30-minute heating period will begin.

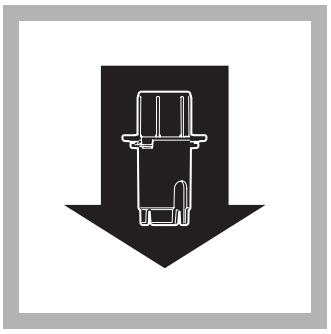


8. When the timer beeps, carefully remove the hot vial from the reactor. Place it in a test tube rack and cool to room temperature.



9. Use a TenSette Pipet to add 2 mL of 1.54 N Sodium Hydroxide Standard Solution to the vial. Cap and mix.

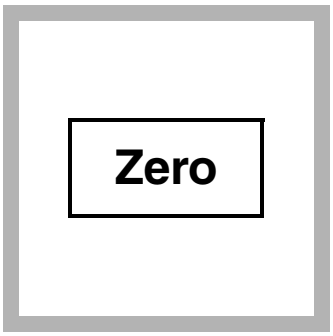
10. Wipe the outside of the vial with a damp cloth followed by a dry one, to remove fingerprints or other marks.



11. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

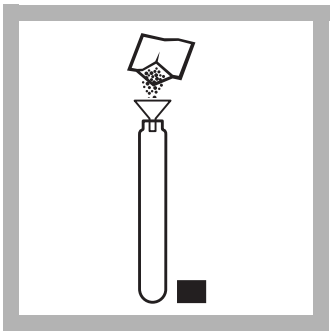
Place the vial into the adapter.



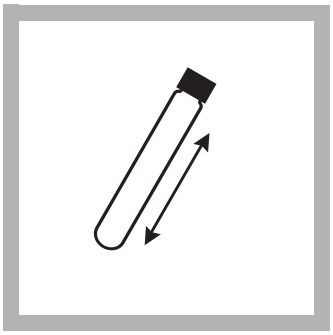
12. Touch **Zero**.

The display will show:

0.00 mg/L PO_4^{3-}

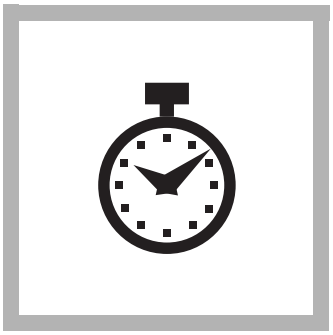


13. Use a funnel to add the contents of one PhosVer 3 Powder Pillow to the vial.



14. Cap tightly and shake to mix for 10–15 seconds.

The powder will not dissolve completely.



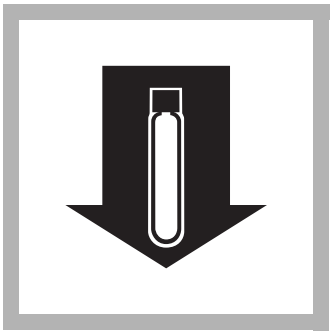
15. Touch the timer icon.

Touch **OK**.

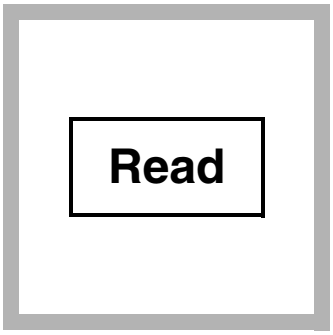
A two-minute reaction period will begin.

Read the sample within 2–8 minutes after the timer beeps.

16. After the timer beeps, wipe the outside of the vial with a damp cloth followed by a dry one, to remove fingerprints or other marks.



17. Place the prepared sample vial into the cell adapter.



18. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Sulfide	Greater than 90 mg/L
Turbidity (large amounts) or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L

Sample Collection, Storage, and Preservation

Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze the samples immediately for the most reliable results. If prompt analysis is not possible, samples may be preserved up to 28 days by adjusting the pH to 2 or less with concentrated Sulfuric Acid (about 2 mL per liter) (Cat. No. 979-49) and storing at 4 °C. Warm the sample to room temperature and neutralize with 5.0 N Sodium Hydroxide (Cat. No. 2450-53) before analysis. Correct for volume additions: see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Section 3.2.2 Standard Additions* on page *32* for more information.
5. Open a Phosphate 10-mL Ampule Standard, 50-mg/L as PO_4^{3-}.

6. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
7. Analyze each standard addition sample as described above (use a 5-mL aliquot of the spiked sample as the sample). Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 1.0-mg/L phosphate standard solution in place of the sample. Perform the procedure as describe above.
2. To adjust the calibration curve using the reading obtained with the 1.0-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision
Standard: 3.00 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
536	2.90–3.10 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.06 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Phosphates present in organic and condensed inorganic forms (meta-, pyro-, or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Phosphorus, Total

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Total Phosphorus Test 'N Tube™ Reagent Set		50 tests	27426-45
Includes:			
PhosVer™ 3 Phosphate Reagent Powder Pillows	1	50/pkg	21060-46
Potassium Persulfate Powder Pillows	1	50/pkg	20847-66
Sodium Hydroxide Solution, 1.54 N	2 mL	100 mL	27430-42
Total and Acid Hydrolyzable Test Vials	1	50/pkg	*
Water, deionized		4 liters	272-42

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
COD Reactor, 115/230 VAC (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 VAC (Europe)	1	each	45600-02
Funnel, micro	1	each	25843-35
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	1	1000/pkg	21856-28
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1–3	each	18641-00

Required Standards

Phosphate Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as PO_4^{3-}		16/pkg	171-10
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}		500 mL	2569-49

*Not sold separately.

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WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

DR/2400 Phosphorus, Total

Method 10127

Molybdovanadate Method with Acid Persulfate Digestion*

Test 'N Tube™ Vials

HR (1.0 to 100.0 mg/L PO_4^{3-})

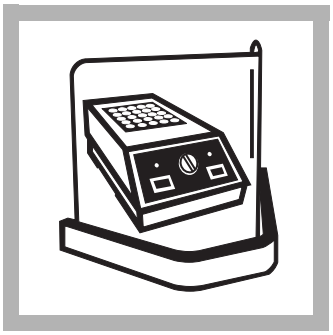
Scope and Application: For water and wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- Place a safety shield in front of the COD reactor to prevent injury if splattering occurs.
- Reagent blanks can be used more than once, but should not be used more than one day.
- The final samples will contain molybdenum. In addition, the final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Consult the Material Data Safety Data Sheet for information specific to the reagents used.

Test 'N Tube — Method 10127

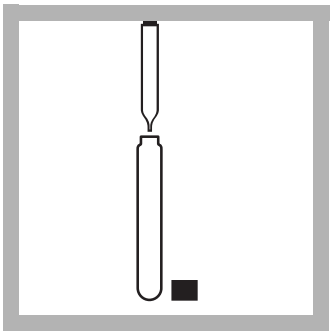


1. Turn on the COD Reactor. Heat to 150 °C. Place the safety shield in front of the reactor.

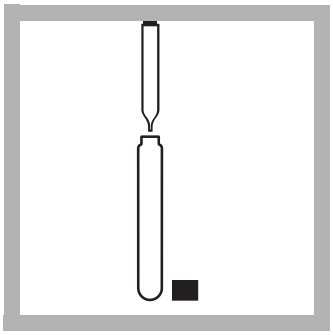
2. Touch **Hach Programs**.

Select program **541 P Total HR TNT**.

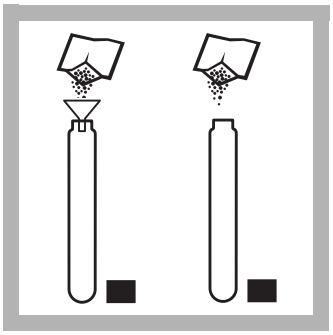
Touch **Start**.



3. Use a TenSette® Pipet to add 5.0 mL of deionized water to a Total Phosphorus Test 'N Tube Vial (the blank).

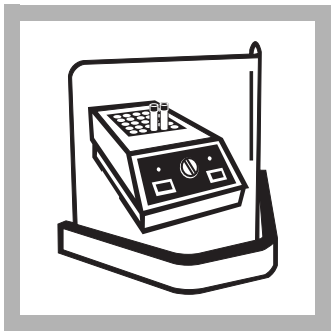


4. Use a TenSette Pipet to add 5.0 mL of sample to a Total Phosphorus Test 'N Tube Vial (the sample).

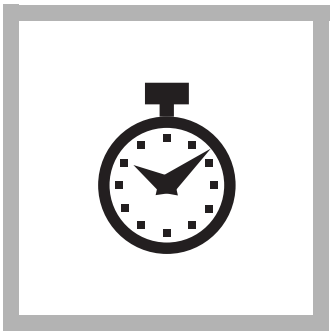


5. Use a funnel to add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to each vial.

Cap tightly and shake to dissolve.



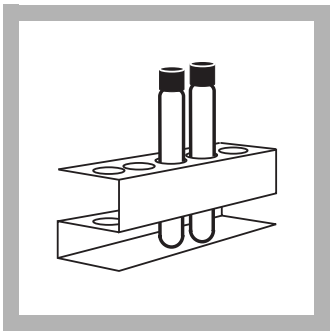
6. Place the vials in the COD Reactor.



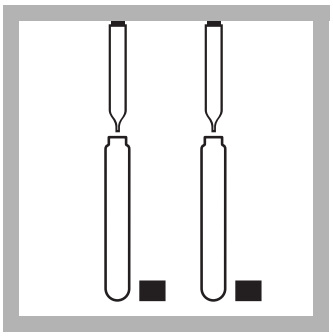
7. Touch the timer icon.

Touch **OK**.

A 30-minute heating period will begin.

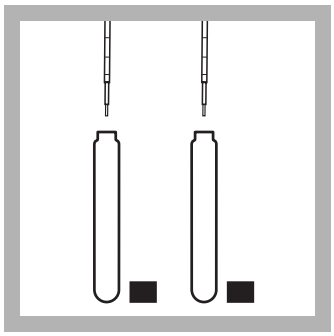


8. After the timer beeps, carefully remove the hot vials from the reactor. Place them in a test tube rack and allow to cool to room temperature (18–25 °C).



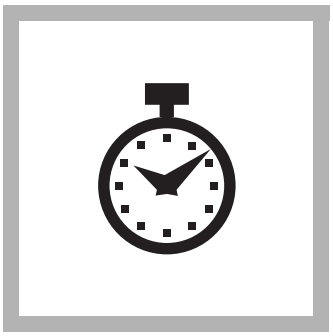
9. Use a TenSette Pipet to add 2.0 mL of 1.54 N sodium hydroxide to each vial.

Cap and invert to mix.



10. Use a polyethylene dropper to add 0.5 mL of Molybdovanadate Reagent to each vial.

Cap and invert to mix.

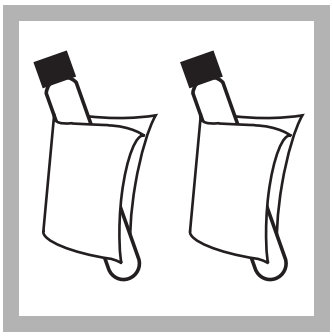


11. Touch the timer icon.

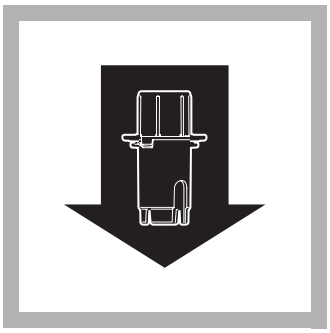
Touch **OK**.

A 7-minute reaction period will begin.

Read the sample within seven to nine minutes after adding the Molybdovanadate Reagent.



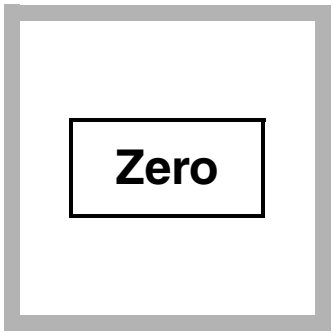
12. Wipe the vials with a damp towel, followed by a dry one, to remove fingerprints or other marks.



13. Install the 16-mm adapter.

Note: *See Section 2.6 in the Instrument Manual for installation details.*

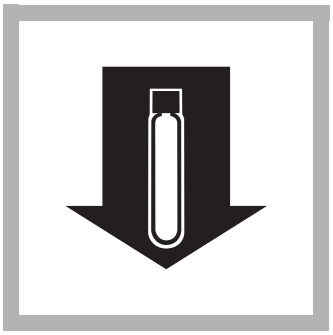
Place the sample vial into the adapter.



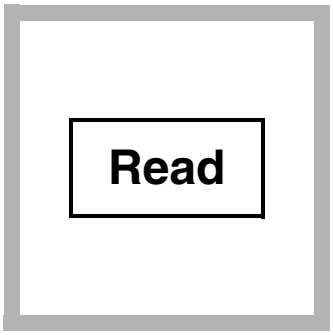
14. Touch **Zero**.

The display will show:

0.0 mg/L PO_4^{3-}



15. Place the prepared sample into the cell adapter.



16. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

Large amounts of sample turbidity may cause inconsistent results in the test because the acid present in the reagents may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.

The following may interfere when present in concentrations exceeding these listed below:

Interfering Substance	Interference Levels and Treatments
Arsenate	Causes positive interference if the sample is heated.*
Iron, ferrous	Blue color caused by ferrous iron does not interfere if iron concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Causes positive interference if the sample is heated.
Extreme pH or highly buffered samples	May exceed buffering capacity of the reagents. See *Section 3.3 Interferences* on page *41*. Samples may require pretreatment. Sample pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause a negative interference.
Temperature, Cold (less than 18 °C)	Cause a negative interference.
Temperature, Hot (greater than 25 °C)	Causes a positive interference. Post-digestion samples should be brought to room temperature (18–25 °C) before the addition of the Molybdovanadate Reagent or sodium hydroxide.
The following do not interfere in concentrations up to 1000 mg/L: Pyrophosphate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, salicylate, Al^{3+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Li^{+}, Na^{+}, K^{+}, NH_4^{+}, Cd^{2+}, Mn^{2+}, NO_3^{-}, NO_2^{-}, SO_4^{2-}, SO_3^{2-}, Pb^{2+}, Hg^{+}, Hg^{2+}, Sn^{2+}, Cu^{2+}, Ni^{2+}, Ag^{+}, U^{4+}, Zr^{4+}, AsO_3^{-}, Br^{-}, CO_3^{2-}, ClO_4^{-}, CN^{-}, IO_3^{-}, SiO_4^{4-}.	

* Gentle warming of the sample to reach room temperature will not cause this substance to interfere.

Sampling and Storage

Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution (Cat. No. 884-49) and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning the glassware used in this test.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples up to 28 days by adjusting the pH to 2 or less with concentrated Sulfuric Acid (about 2 mL per liter) (Cat. No. 979-49) and storing at 4 °C. Warm the sample to room temperature and neutralize with 5.0 N Sodium Hydroxide (Cat. No. 2450-53) before analysis. Correct for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Snap the neck off a 10-mL Voluette® Ampule of Phosphate Standard Solution, 500 mg/L as PO_4^{3-}.
6. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 10 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively to each cylinder. Mix well.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery or an increase of 5 mg/L PO_4^{3-} for each 0.1 mL of standard added.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 50-mg/L Phosphate standard in place of the sample. Perform the procedure as described.
2. To adjust the calibration curve using the reading obtained with the 50-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.

3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 50 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
541	49.5–50.5 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	1.0 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Phosphates present in organic and condensed inorganic forms (meta-, pyro-, or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. In the presence of vanadium, yellow molybdovanadophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration. Test results are measured at 420 nm.

Phosphorus, Total

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Total High Range Phosphorus Test 'N Tube™ Reagent Set		50 vials	27672-45
Includes:			
(1) Molybdovanadate Reagent	0.5 mL	25 mL	20760-26
(1) Potassium Persulfate powder Pillows	1	50/pkg	20847-66
(1) Sodium Hydroxide Solution, 1.54 N	2 mL	100 mL	27430-42
(1) Total Phosphorus Test Vials	1	50/pkg	*
(2) Water, deionized		100 mL	272-42

Required Apparatus

Adapter, 16-mm Cell	1	each	59457-00
COD Reactor, 115/230 VAC (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 VAC (Europe)	1	each	45600-02
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	1	250/pkg	21997-25
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1–3	each	18641-00

Required Standards

Phosphate Standard Solution, Voluette® ampule, 500-mg/L as PO_4^{3-}, 10-mL		16/pkg	14242-10
Phosphate Standard Solution, 50-mg/L as PO_4^{3-}		500 mL	171-49

* Not available separately.

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3/04 1ed

Phosphorus, Total

Ascorbic Acid Method with Acid Persulfate Digestion

UniCell™ Vials

(1.5 to 15.0 mg/L PO_4^{3-})

Scope and Application: For water, wastewater, boiler water, surface water, and process control

Tips and Techniques

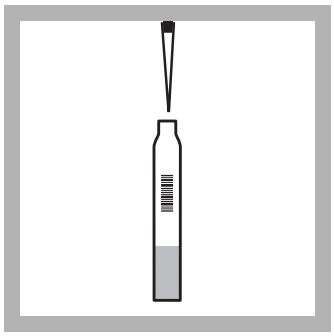
- Adjust the pH of preserved samples to between pH 2–10 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel followed by a dry one.
- Use the standard solution or standard addition method to verify results (see *Accuracy Check on page 4*).
- See *Adjusting the Standard Curve on page 40* for information on adjusting the calibration curve.
- Make sure the temperature of the water sample and the sample vial is between 18–20 °C. Temperatures outside of this range will affect the color of the reaction and therefore may give inaccurate results.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

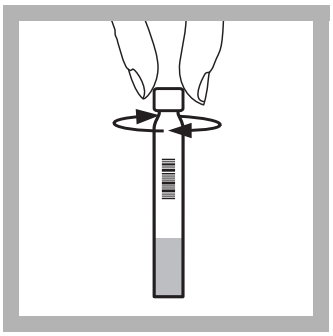
1. Touch **Hach Programs**.

Select program **806 Phosphate HCT 121**.

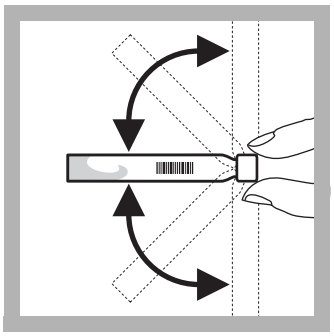
Touch **Start**.



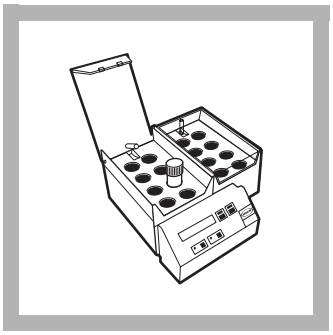
2. Pipet 1.0 mL of sample into a sample vial.



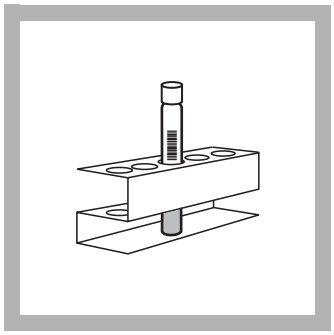
3. Screw a **blue** UniCap A (HCT 121 A) onto the sample vial.



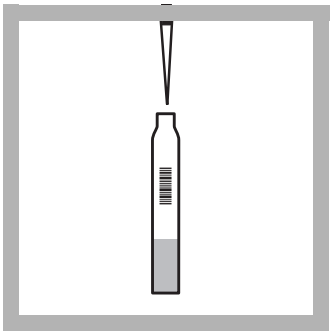
4. Invert the sample vial repeatedly until the solid reagent in the cap is dissolved.



5. Place the vial into the Reactor Block. Heat at 100 °C for 60 minutes.

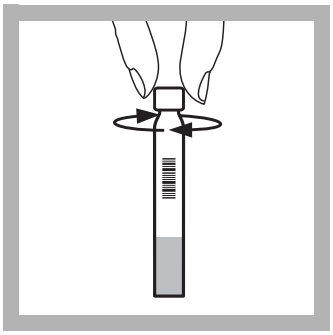


6. After the 60-minute heating period, carefully remove the hot vial from the reactor. Place it in a test tube rack and cool to room temperature.

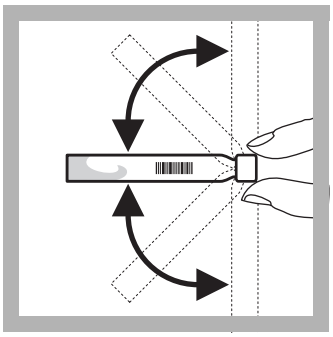


7. Pipet 0.4 mL of Reagent B (HCT 121/122 B) into the cooled sample vial.

Close the Reagent B bottle **immediately** after use.



8. Screw a **light green** UniCap C (HCT 121 C) onto the sample vial.

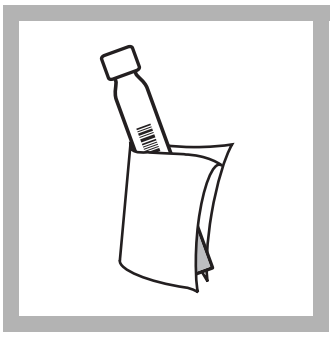


9. Invert the sample vial repeatedly until the solid reagent in the cap is dissolved.

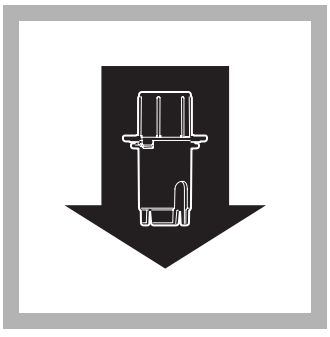
10. Touch the timer icon.

Touch **OK**.

A 10-minute reaction period will begin.



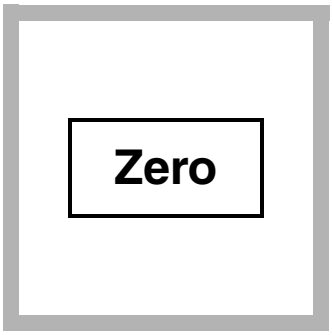
11. Wipe the outside of the zero (white cap) and sample vials with a damp cloth followed by a dry one, to remove fingerprints or other marks.



12. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

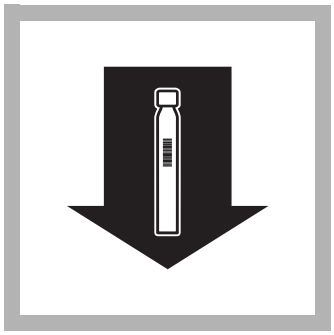
When the timer beeps, place the zero vial into the cell adapter.



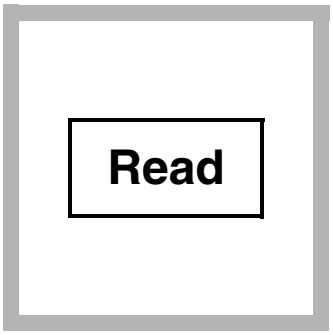
13. Touch **Zero**.

The display will show:

0.0 mg/L PO_4^{3-}
Underrange



14. Place the prepared sample vial into the cell adapter.



15. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	No interference up to:
SO_4^{2-}	20 g/L
Cl^-	10 g/L
K^+, Na^+	4 g/L
Ca^{2+}	1 g/L
Mg^{2+}	400 mg/L
Co^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, NO_2^-, Cd^{2+}, NH_4^+, Mn^{2+}, Al^{3+}, CO_3^{2-}	200 mg/L
I^-	100 mg/L
SiO_2	50 mg/L
Hg^{2+}	40 mg/L
Sn^{2+}	25 mg/L
Pb^{2+}	20 mg/L
Ag^+, Cr^{3+}	10 mg/L
Cr^{6+}	1 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Store in a cool dry place in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

For wastewater samples — If prompt analysis is impossible, preserve samples* by adjusting the pH to 2 or less with Sulfuric Acid, 5.25 N (Cat. No. 2449-00) and storing the sample at 4 °C for up to 28 days. The pH can be checked using pH Paper (Cat. No. 391-33). Do not use mercury compounds as preservatives. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *3.2.2 Standard Additions* for more information.
5. Snap the neck off a 10-mL Voluette® Ampule of Phosphate Standard Solution, 500-mg/L as PO_4^{3-}.
6. Prepare three sample spikes. Fill three mixing cylinders with 100 mL of sample. Use a pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of standard, respectively, to each cylinder. Mix well.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 10-mg/L PO_4^{3-} standard solution listed under *Optional Standards*. Perform the phosphate procedure as described.
2. To adjust the calibration curve using the reading obtained with the 10-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

* See 40 CFR 136.3

Method Performance

Precision
Standard: 7.5 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
806	7.2–7.8 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
806	Entire range	0.010	0.2 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Phosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex. Ascorbic acid reduces this complex to phosphomolybdenum blue. Total phosphate measurements include a digestion step while orthophosphate measurements exclude digestion. Test results are measured at 880 nm.

Required Reagents

Description	Unit	Cat. No.
Phosphate - PO_4^{3-}, LR, UniCell™ HCT 121	23/pkg	HCT 121

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Cylinder, mixing, 100-mL graduated	each	20886-42
Digital Reactor Block 100	each	DRB 100
Pipettor, Jencons, 100–1000 µL	each	27949-00
Rack, cooling, test tube	each	18641-00
Tips, replacement for 27949-00	400/pkg	27950-00

Optional Standards

Hydrochloric Acid Solution, 1:1	500 mL	884-49
Phosphate Standard, 10-mL Voluette Ampule, 500-mg/L as PO_4	16/pkg	14242-10
Phosphate Standard, 10-mg/L as PO_4	946 mL	14204-16

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Phosphorus, Total

Ascorbic Acid Method with Acid Persulfate Digestion

UniCell™ Vials **(6.0 to 60.0 mg/L PO_4^{3-})**

Scope and Application: For water, wastewater, boiler water, surface water, and process control

Tips and Techniques

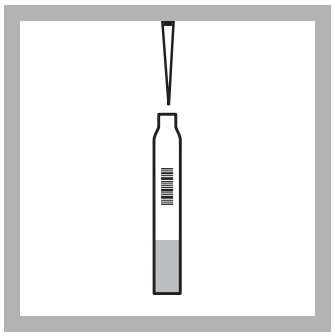
- Adjust the pH of preserved samples to between pH 2–10 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results (see *Accuracy Check on page 3*).
- See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for information on adjusting the calibration curve.
- Make sure the temperature of the water sample and the sample vial is between 18–20 °C. Temperatures outside of this range will affect the color of the reaction and may give inaccurate results.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

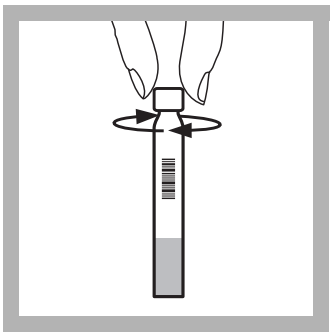
1. Touch **Hach Programs**.

Select program **804 Phosphate, HCT 122**.

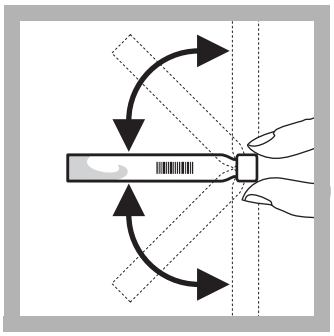
Touch **Start**.



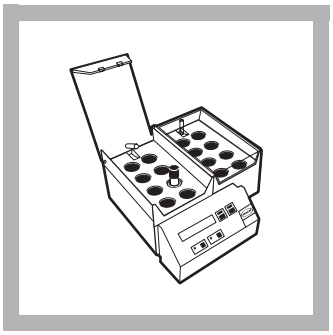
2. Pipet 0.4 mL of sample into a sample vial.



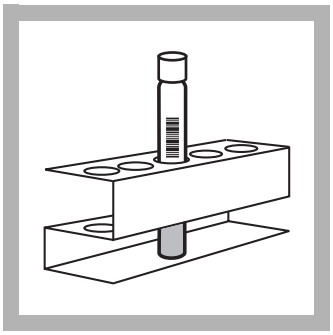
3. Screw an **orange** UniCap A (HCT 122 A) onto the sample vial.



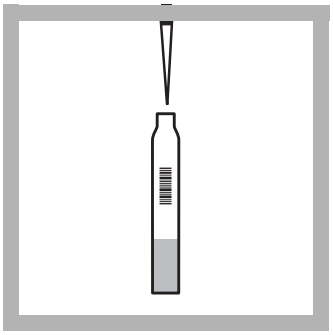
4. Invert the sample vial repeatedly until the solid reagent in the cap is dissolved.



5. Place the vial into the Reactor Block. Heat at 100 °C for 60 minutes.

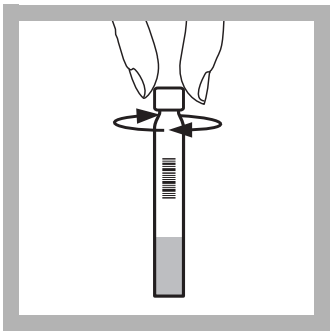


6. After the 60-minute heating period, carefully remove the hot vial from the reactor. Place it in a tube rack and cool to room temperature (15–25 °C).

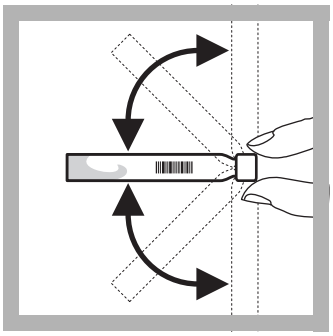


7. Pipet 0.5 mL of Reagent B (HCT 121/122 B) into the cooled sample vial.

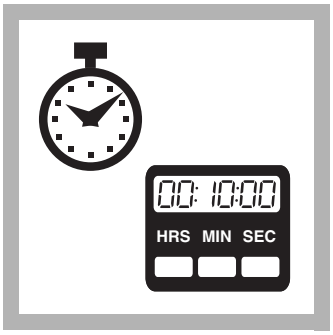
Close the Reagent B bottle **immediately** after use.



8. Screw a **grey** UniCap C (HCT 122 C) onto the sample vial.



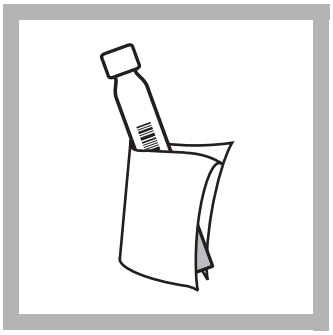
9. Invert the sample vial repeatedly until the solid reagent in the cap is dissolved.



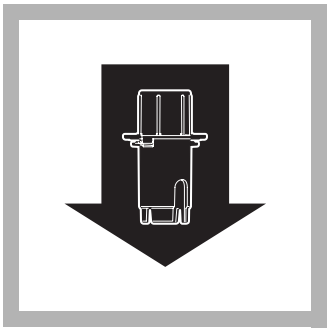
10. Touch the timer icon.

Touch **OK**.

A 10-minute reaction period will begin.



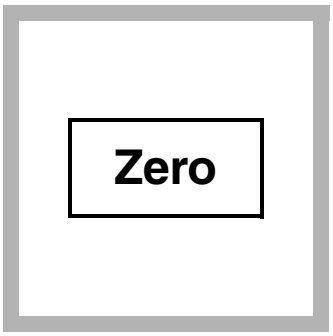
11. Wipe the outside of the zero (**white** cap) and sample vials with a damp cloth followed by a dry one, to remove fingerprints or other marks.



12. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

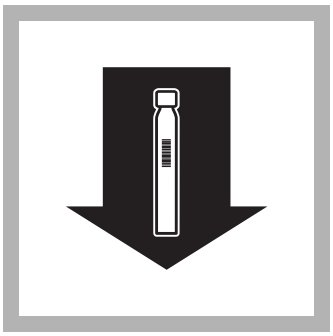
When the timer beeps, place the zero vial into the cell adapter.



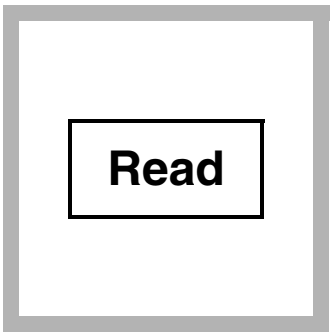
13. Touch **Zero**.

The display will show:

0.0 mg/L PO_4^{3-}
Underrange



14. Place the prepared sample vial into the cell adapter.



15. Touch **Read**.

Results will appear in mg/L PO_4^{3-}.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	No interference up to:
SO_4^{2-}	5000 mg/L
Cl^-	2000 mg/L
K^+, Na^+, Ca^{2+}	1000 mg/L
Mg^{2+}, NO_3^-	500 mg/L
Co^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Cr^{3+}, I^-, NO_2^-, Cd^{2+}, Sn^{2+}, NH_4^+, Mn^{2+}, Al^{3+}, Hg^{2+}, Pb^{2+}, SiO_2	50 mg/L
Ag^+	25 mg/L
Cr^{6+}	5 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Store in a cool dry place in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

For wastewater samples — If prompt analysis is impossible, preserve samples* by adjusting the pH to 2 or less with Sulfuric Acid, 5.25 N (Cat. No. 2449-00) and storing the sample at 4 °C for up to 28 days. The pH can be checked using pH paper (Cat. No. 391-33). Do not use mercury compounds as preservatives. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
3. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
4. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
5. Snap the neck off a 10-mL Voluette® Ampule of Phosphate Standard Solution, 500-mg/L as PO_4^{3-}.

* See 40 CFR 136.3

6. Prepare three sample spikes. Fill three mixing cylinders with 25 mL of sample. Use a pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each cylinder. Mix well.
7. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use a 30-mg/L Phosphate Standard Solution listed under *Optional Standards*. Perform the phosphate procedure as described.
2. To adjust the calibration curve using the reading obtained with the 30-mg/L PO_4^{3-} Phosphate Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision
Standard: 30.0 mg/L PO_4^{3-}

Program	95% Confidence Limits of Distribution
804	29.5–30.5 mg/L PO_4^{3-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
804	Entire range	0.010	0.5 mg/L PO_4^{3-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Phosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex. Ascorbic acid reduces this complex to phosphomolybdenum blue. Test results are measured at 880 nm.

Required Reagents

Description	Unit	Cat. No.
Phosphate - PO_4^{3-}, UniCell™ HCT 122	23/pkg	HCT 122

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Cylinder, mixing, graduated 25-mL	each	20886-40
Digital Reactor Block 100	each	DRB 100
Pipettor, Jencons, 100–1000 µL	each	27949-00
Rack, cooling, test tube	each	18641-00
Tips, replacement for 27949-00	400/pkg	27950-00

Optional Standards

Hydrochloric Acid Solution 1:1	500 mL	884-49
Phosphate Standard, 10-mL Voluette® Ampule, 500-mg/L as PO_4	16/pkg	14242-10
Phosphate Standard, 30-mg/L as PO_4	946 mL	14367-16

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3/04 1ed

Potassium

Method 8049

Tetraphenylborate Method

Powder Pillows

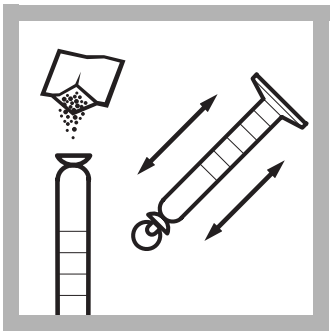
(0.1 to 7.0 mg/L)

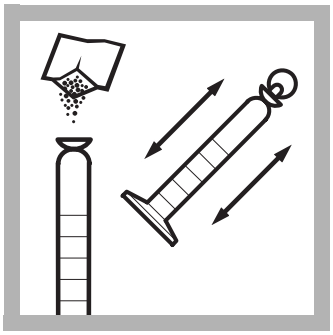
Scope and Application: For water, wastewater, and seawater

Tips and Techniques

- Program # 905 has a calibration curve for potassium; however, due to potential variation between lots of Potassium 3 Reagent, perform a new calibration for each lot of reagent to obtain best accuracy. Prepare and store the calibration as directed under Calibration on page *3*.
- Some variations of the calibration procedure are possible. See the *Instrument Manual* for details.
- Filter highly colored or turbid samples before analysis.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- The final samples are highly acidic. Neutralize to pH 6–9 and flush to drain for disposal. For more information on pollution prevention and waste management, refer to *Waste Management and Safety on page 55*.
- After the test, clean the cells with soap and a brush.

Powder Pillows — Method 8049





1. Touch

User Program.

Select the stored program number for potassium.

Touch **Start**.

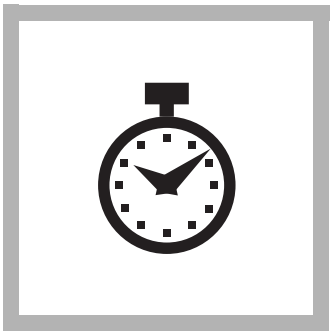
Note: *When performing this procedure for the first time the instrument must be programmed. See User Programming on page 4.*

2. Fill a graduated mixing cylinder with 25 mL of sample.

3. Add the contents of one Potassium 1 Reagent Pillow. Add the contents of one Potassium 2 Reagent Pillow. Stopper and invert several times to mix.

4. Add the contents of one Potassium 3 Reagent Pillow after the solution clears. Stopper and shake the solution for 30 seconds.

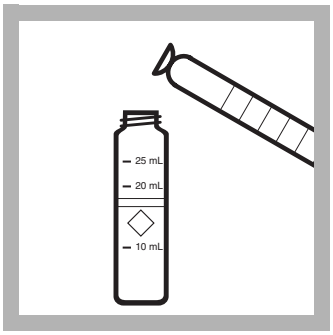
A white turbidity will form if potassium is present.



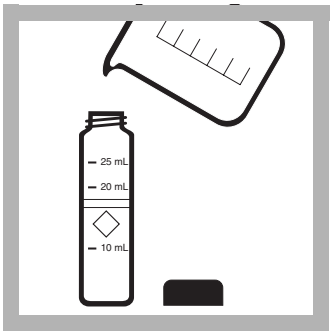
5. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin.

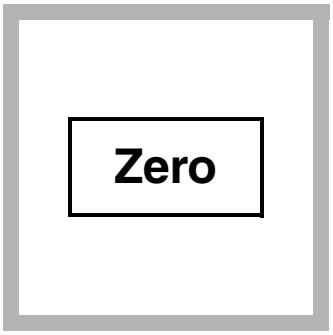


6. Pour the solution from the cylinder into a 25-mL sample cell (this is the prepared sample).



7. When the timer beeps, fill the second sample cell with 25 mL of sample (this is the blank).

8. Wipe the blank and place it into the cell holder.

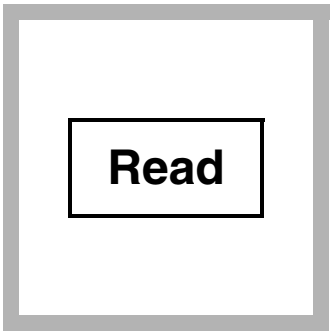


9. Touch **Zero**.

The display will show:

0.0 mg/L K

10. Within seven minutes after the timer beeps, wipe the prepared sample and place it into the cell holder.



11. Touch **Read**.

Results will appear in mg/L K.

Interferences

The substances listed below have been tested and will not interfere at or below the levels stated. If these substances are present at higher levels, conduct interference studies at the higher levels to determine if the substance interferes.

Interfering Substance	Interference Levels and Treatments
Ammonium Nitrogen	15 mg/L as N
Calcium	7000 mg/L as $CaCO_3$
Chloride	15,000 mg/L
Magnesium	6000 mg/L as $CaCO_3$

Sample Collection, Preservation, and Storage

Collect samples in acid-washed plastic bottles. Adjust the pH to 2 or less with Nitric Acid (about 2 mL per liter) (Cat. No. 2540-49). Preserved samples may be stored at least six months at room temperature. Before analysis, adjust the pH to 4–5 with 5.0 N Sodium Hydroxide (Cat. No. 2450-26). Do not measure pH in the sample container with a pH electrode, as this will introduce potassium from the filling solution. Use pH Paper (Cat. No. 391-33) or pour off sample and test pH in a separate beaker. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the DR/2400 instrument manual for more information.
4. Snap the neck off a Potassium Voluette® Ampule Standard, 250-mg/L K.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Calibration

Standard Preparation

An approximate calibration curve is preprogrammed within Program 905. For improved accuracy, a new calibration should be performed with each new lot of reagents. Prepare calibration standards containing 1, 2, 3, 4, 5, 6, 7, and 8 mg/L potassium as follows:

1. Into eight different 100-mL Class A volumetric flasks, pipet 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 mL of the 100-mg/L Potassium Standard Solution using class A glassware or TenSette Pipet.
2. Dilute to the mark with deionized water. Mix thoroughly.
3. Use deionized water for the 0-mg/L potassium standard.

User Programming

1. Touch **User Programs** on the main menu.
2. If you have not performed a potassium calibration before, touch **Program Options** and **New Program**.
3. Assign a program number to the potassium user program. Touch **OK**.
4. Select **Wavelength**. Touch **Edit**. Enter **650**. Touch **OK**.
5. An editable list of parameters will appear on the screen. Touch **Calibration: C = a + bA**. Touch **Edit**. Touch **Curve Fit**.
6. Perform steps 3 through 6 of the procedure, substituting the potassium standards for the sample.
7. Fill a 25-mL cell with deionized water and place it in the cell holder. Touch **+**. Input a concentration of 0.0 mg/L. Touch **OK**. Touch **Zero**.
8. Touch the down arrow and then touch **+** to add the next standard. Key in the concentration of the standard. Touch **OK.** Touch **Read** for the standard absorbance. Repeat this step until all the standards have been read.
9. When all the absorbances have been read, touch **Graph** to view the calibration graph. Touch **Force 0** to force the curve through zero. Touch **Next Formula** until the formula displayed above the graph gives the best curve fit. Touch **Done** to return to the calibration screen. Touch **OK** to return to the parameter list.
10. Edit the following parameters:

Name: **Potassium**	Wavelength: **650 nm**	Lower Limit: **0.0**
Units: **mg/L**	Resolution **0.1**	Timer 1: **3:00**
Chemical Form1: **K**	Upper Limit: **7.0**	

11. When the parameters have been edited, touch **Done**. Touch **Yes** to save the program. The information is now stored as a User Calibration, and the program is ready for use.

Method Performance

Precision

Standard: 5.0 mg/L K

Program	95% Confidence Limits of Distribution
905	4.6–5.4 mg/L K

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.1 mg/L K

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Potassium in the sample reacts with sodium tetraphenylborate to form potassium tetraphenylborate, an insoluble white solid. The amount of turbidity produced is proportional to the potassium concentration. Test results are measured at 650.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Potassium Reagent Set (100 tests)			24591-00
Includes:			
(4) Potassium 1 Reagent Powder Pillows	1 pillow	25/pkg	14321-98
(4) Potassium 2 Reagent Powder Pillows	1 pillow	25/pkg	14322-98
(1) Potassium 3 Reagent Powder Pillows	1 pillow	100/pkg	14323-99
Potassium Standard Solution, 100-mg/L	varies	500 mL	23517-49
Water, deionized		4 liters	272-56

Required Apparatus

Clippers, for opening powder pillows	1	each	968-00
Cylinder, mixing, graduated, 25-mL	1	each	1896-40
Flask, volumetric, 100-mL, Class A	8	each	14574-42
Pipet, TenSette®, 1–10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	varies	50/pkg	21997-96
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Potassium Standard Solution, 100-mg/L	varies	500 mL	23517-49
Potassium Standard Solution, 10-mL Voluette® Ampule, 250-mg/L		16/pkg	14790-10
Water, deionized		4 liters	272-56

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3/04 1ed

DR/2400

Quaternary Ammonium Compounds

Method 8337

Direct Binary Complex Method

Powder Pillows

(0.2 to 5.0 mg/L as CTAB)

Scope and Application: For cooling tower water and pool/spa water

Powder Pillows **Method 8337**

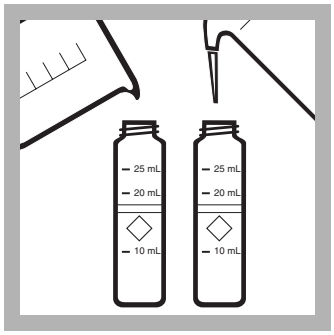
1. Touch

Hach Programs.

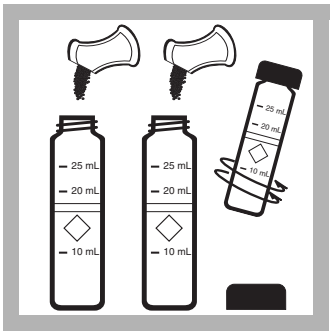
Select program

401 QAC.

Touch **Start**.



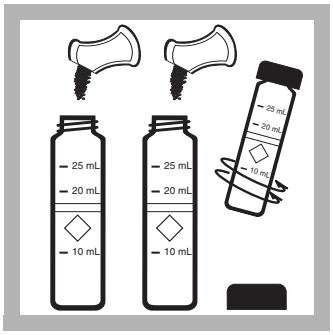
2. Fill one sample cell with 25 mL of deionized water (the blank) and another sample cell with 25 mL of sample (the prepared sample).



3. Add the contents of one QAC Reagent 1 Powder Pillow to each sample cell.

Swirl the sample cells to dissolve the reagent.

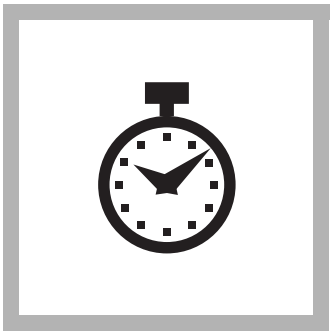
Do not shake! Shaking creates air bubbles that interfere with test results.



4. Add the contents of one QAC Reagent 2 Powder Pillow to each sample cell.

Swirl the sample cells to dissolve the reagent. Do not shake.

A purple color will form if quaternary ammonium compound is present.

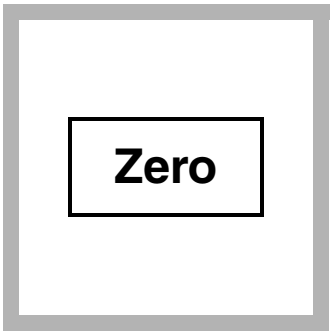


5. Touch the timer icon.

Touch **OK**.

A two-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0.0 mg/L CTAB

8. Place the prepared sample into the cell holder.

Touch **Read**.

9. Results will appear in mg/L CTAB (cetyl-trimethylammonium bromide).

Interferences

Interference studies were conducted by preparing a CTAB standard solution of approximately 3 mg/L as well as a solution of the potential interference. The constituent was said to interfere when the resulting concentration changed by 10%.

Interfering Substance	Interference Levels and Treatments
Calcium (as $CaCO_3$)	Positive interference above 1350 mg/L
Chlorine, HOCl and OCl^-	Positive interference above 7 mg/L
Cyanuric acid	Negative interference above 70 mg/L
Igepal™ nonionic surfactant	Positive interference above 3 mg/L
Iodine, I_3^-	Positive interference above 3 mg/L
Iron, Fe^{3+}	Positive interference above 80 mg/L
Liquimine™ 14–P, filming amine	Positive interference above 1825 mg/L
Magnesium, Mg^{2+}	Positive interference above 1350 mg/L
Niaproof™ anionic surfactant	Negative interference above 11 mg/L
Polyacrylic acid	Negative interference above 16 mg/L
Sodium lauryl sulfate	Negative interference above 8 mg/L
Sodium polyphosphate	Positive interference above 1325 mg/L
Tribenzylamine	Positive interference above 7 mg/L
Triton X-100™ nonionic surfactant	Positive interference above 4 mg/L
Urea	Positive interference above 8 mg/L
Highly buffered samples or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment. Adjust the sample pH between 3 and 5 by using a pH meter or pH paper and adding dropwise an appropriate amount of acid or base such as 1.0 N Sulfuric Acid Standard Solution (Cat. No. 1270-26) or 1.0 N Sodium Hydroxide Standard Solution (Cat. No. 1045-32). If significant volumes of acid or base are used, a volume correction should be made. See *Section 3.1.3 Correcting for Volume Additions* on page *29.*

Non-interfering Substance	Highest Concentration Tested (mg/L)
Silica, SiO_2	400
Potassium alum, $AlKS_2O_8$	500
Sodium thiosulfate, $Na_2S_2O_3$	30

After several samples have been analyzed, the sample cells may exhibit a build-up of a pink or purple color. A rinse with 1.0 N Sodium Hydroxide Solution (Cat. No. 1045-53) followed by a Alconox™ detergent (Cat. No. 20880-00) wash and deionized water rinse will eliminate the build-up when it occurs.

Sample Collection, Storage, and Preservation

Collect samples in glass bottles that have been rinsed several times with sample before final sample filling. Do not use plastic containers as plastic adsorbs QACs. Acidify the sample to a pH of less than 2. Store at 4 ±2 °C.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a QAC Standard Solution, 100-mg/L CTAB.
5. Prepare three sample spikes. Fill three sample cells with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery. Each 0.1 mL should increase the QAC reading by 0.4 mg/L CTAB.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 5.0-mg/L CTAB standard solution as follows:

1. Pipet 5.0 mL of QAC Standard, 100-mg/L as CTAB, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Mix well. Prepare this solution daily. Perform the quaternary ammonium compound procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 5.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision
Standard: 1.8 mg/L CTAB

Program	95% Confidence Limits of Distribution
401	1.4–2.2 mg/L CTAB

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.04 mg/L CTAB

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The test method makes use of a colorimetric chemistry in which a quaternary ammonium compound reacts with an indicator to produce a color change from pale pink to vivid purple. The test is conducted in a stabilized, acid-buffered solution containing a masking agent to eliminate potential interferences. This test is applicable to the monitoring of QACs in swimming pools and cooling towers. Test results are measured at 575 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Quaternary Ammonium Compounds Reagent Set (100 tests)			24592-00
Includes:			
(4) QAC Reagent 1 Powder Pillows	2 pillows	50/pkg	24010-66
(8) QAC Reagent 2 Powder Pillows	2 pillows	25/pkg	24012-68

Required Apparatus

Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 25-mL	1	each	508-40
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

QAC Standard Solution, 100-mg/L as CTAB		100 mL	24153-42
Water, deionized		4 liters	272-56

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Method 8194

Diaminobenzidine Method*
(0.01 to 1.00 mg/L)

Scope and Application: For water and wastewater

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- Distillation is required for determining total selenium. See the *Distillation on page 4* at the end of the procedure. Use the distillate in *step 2*.
- Acetone (Cat. No. 14429-49) is a suitable solvent for removing toluene from glassware after results are measured.
- Toluene (F005) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Water saturated with toluene, toluene solutions, and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent wastes. See *Section 4* on page *55* for more information on proper disposal of these materials.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- If there are visible water bubbles on the bottom of the cell, decant the top portion into a clean, dry 10-mL cell prior to reading the sample.

Method 8194

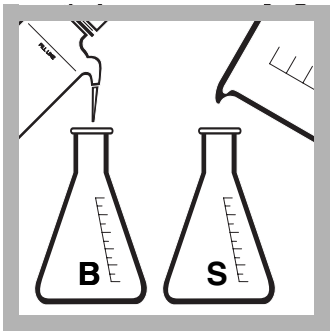
1. Touch

Hach Programs.

Select program

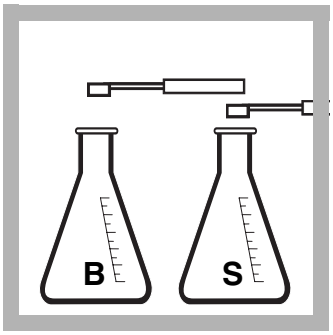
640 Selenium.

Touch **Start**.

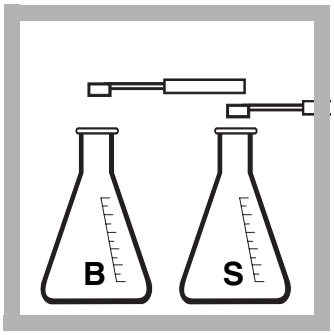


2. Measure 100 mL of deionized water into a 500-mL Erlenmeyer flask. Label the flask "blank".

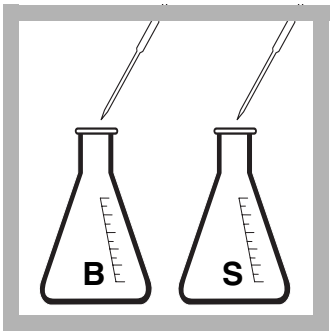
Measure 100 mL of sample into a 500-mL Erlenmeyer flask. Label the flask "sample".



3. Add a 0.2-g spoonful of TitraVer® Hardness Reagent to each flask. Swirl to mix.

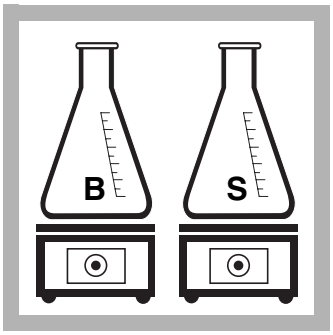


4. Add a 0.05-g spoonful of diaminobenzidene tetrahydrochloride to each flask. Swirl to mix.

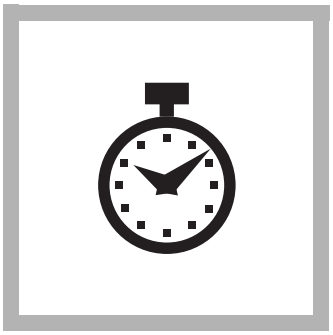


5. If you have not distilled the sample, add 5.0 mL of Buffer Solution, sulfate type, pH 2.0 to each flask. Swirl to mix.

If the sample has been distilled, adjust the pH of the distillate to pH 2.7 ±0.2 using 5 N Sodium Hydroxide Standard Solution (Cat. No. 2450-32). Adjust the blank to pH 2.7 ±0.2 using 5.25 N Sulfuric Acid Standard Solution (Cat. No. 2449-32).



6. Heat each flask on a hot plate. Bring the contents to a gentle boil.

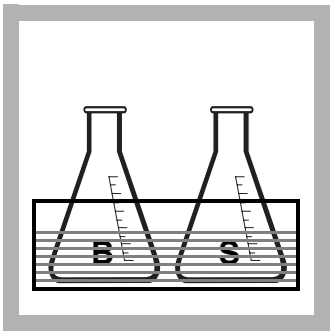


7. Touch the timer icon.

Touch **OK**.

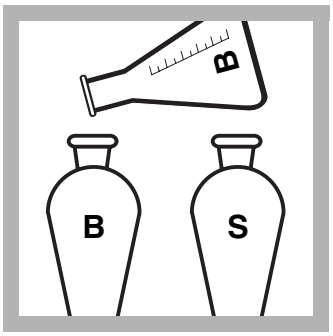
A five-minute reaction period will begin. Continue to boil the contents gently during this time period.

A yellow color will develop if selenium is present.

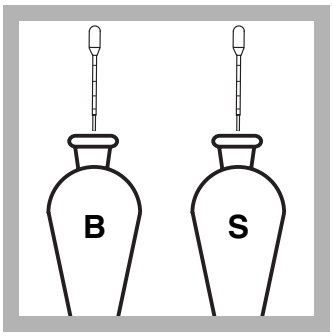


8. When the timer beeps, remove both flasks. Cool to room temperature using a water bath.

Do not boil more than one minute after the timer beeps.



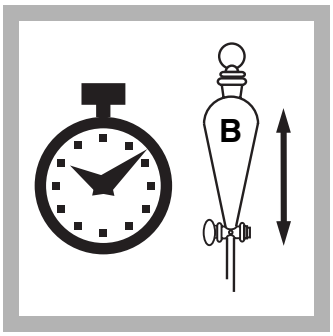
9. Transfer the contents of each flask to separate 250-mL separatory funnels. Label the funnels "blank" and "sample".



10. Add 2.0 mL of 12 N Potassium Hydroxide Standard Solution to each funnel using a calibrated 1.0-mL plastic dropper. Stopper. Shake each funnel to mix.

11. Add 30-mL of toluene to each funnel. Stopper. Swirl and invert each funnel, then open the stopcock to vent the funnel. Close the stopcock. Repeat twice with each funnel.

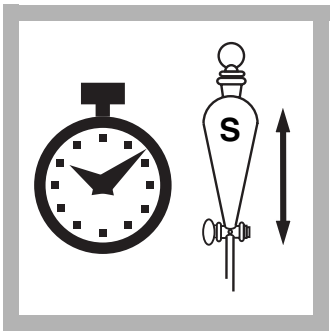
Use toluene only with adequate ventilation.



12. Touch the timer icon.

Touch **OK**.

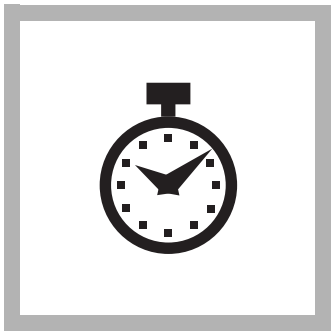
A 30-second reaction period will begin. During this time, vigorously shake the funnel that contains the blank.



13. Touch the timer icon.

Touch **OK**.

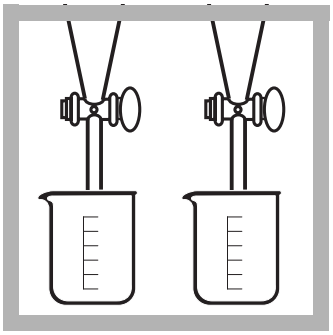
A 30-second reaction period will begin. During this time, vigorously shake the funnel that contains the sample.



14. Touch the timer icon.

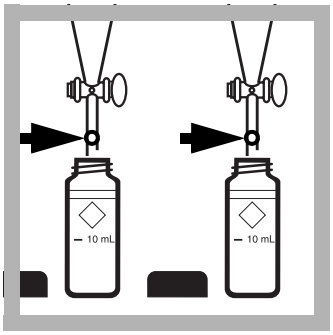
Touch **OK**.

A four-minute reaction period will begin.



15. When the timer beeps, drain the lower water layer from each funnel and discard.

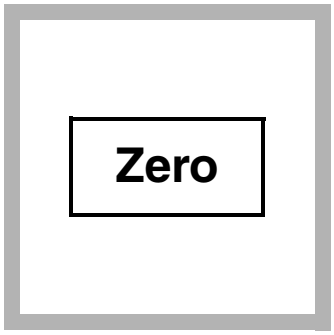
Complete steps 16–20 within five minutes after the timer beeps. The developed color is stable, but should be measured as soon as possible.



16. Insert a cotton plug into the delivery tube of each separatory funnel. Slowly drain the toluene into respective sample cells labeled "blank" and "sample". Fill to the 10 mL line and cap the sample cells.

Note: *Filtering the toluene through dry, absorbent cotton will remove water or suspended particles.*

17. Wipe the blank and place it into the cell holder.

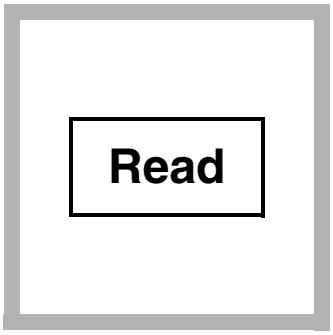


18. Touch **Zero**.

The display will show:

0.00 mg/L Se

19. Wipe the prepared sample and place it into the cell holder.



20. Touch **Read**.

Results will appear in mg/L Se.

Interferences

Interfering Substance	Interference Levels and Treatments
Ferric iron	Up to 2.5 mg/L. Distill sample to eliminate interference.
Manganese	Will not interfere.
Strong oxidizing agents (i.e., iodine, bromine, or chlorine)	Can react with the indicator to give low results. Distill sample to eliminate interference.

Note: *There are no positive inorganic interferences with this method.*

Sample Collection, Storage, and Preservation

Collect samples in clean glass or plastic containers. Adjust the pH to 2 or less with Nitric Acid (Cat. No. 152-49) (about 1.5 mL per liter). Preserved samples can be stored for up to six months at room temperature. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Distillation

Always perform this procedure under a fume hood! This distillation involves the use of a strong acid and oxidizer at high temperatures. To avoid personal injury, observe all laboratory safety precautions when operating the distilling apparatus.

1. Measure 500 mL of sample into a 1000-mL beaker.
2. Add 1 mL of Methyl Orange Indicator Solution. Stir with a glass rod.
3. Use a dropper to add 0.1 N Hydrochloric Acid Standard Solution dropwise until the solution becomes pink. Then add an additional 2 mL.
4. Use a pipet to add 5.0 mL Calcium Chloride Solution. Mix well.
5. Use a dropper to add 1-g/L Potassium Permanganate Standard Solution drop-wise until the solution is purple.
6. Place the beaker on a hot plate. Evaporate the solution to approximately 250 mL. Periodically add 1-g/L Potassium Permanganate Solution to keep the solution purple.
7. Any precipitate formed at this step is manganese dioxide, and may be ignored.
8. Cool the solution. While cooling, set up the distillation apparatus for the general purpose distillation as shown in the *Hach Distillation Manual*.
9. Pour the treated sample solution into the distillation flask. Add a stirring bar to the flask.
10. Pipet 5.0 mL of 0.1 N Sodium Hydroxide Standard Solution into the flask. Turn the stirrer power switch to ON. Set the stir control to 5.
11. Turn on the water and adjust so a constant flow is maintained through the condenser. Set the heat control to 10.
12. When only a few milliliters are left in the distillation flask, turn the power switch off. The distillate in the Erlenmeyer flask may be discarded.
13. **Perform this step under a hood.** When the flask has cooled, add 50 mL of 19.2 N Sulfuric Acid Standard Solution to the flask. Add the contents of one Potassium Bromide Powder Pillow to the flask.

14. Fill a 250-mL beaker to the 75-mL mark with deionized water. Place it under the drip tube. Elevate the beaker with a laboratory jack so the tube extends below the level of the water.
15. Add 1.0 mL of 30% hydrogen peroxide solution to the flask. Turn the stir control to 5 and the heat control to 10. Cap the distillation flask.
16. Heat the distillation flask until the yellow color is gone from the complete distillation apparatus, including the J-tube and condenser. Remove the beaker from under the drip tube.
17. Turn off the heater switch. When the J-tube and condenser have cooled, rinse them with deionized water. Add the washings to the 250-mL beaker. Total volume in the beaker should be approximately 100 mL.
18. Add the Phenol Solution drop-wise to the distilled sample to discharge the bromine color (a white precipitate of tribromophenol will form).
19. Allow the precipitate to settle. Using a dropper, collect about 5 mL of the clear, colorless distillate and transfer to a test tube.
20. Test the solution for completeness of precipitation by adding 2 drops of Phenol Solution. If the solution becomes cloudy or white precipitate forms, residual bromine is still present (proceed to next step). If no cloudiness occurs, the sample is ready for analysis.
21. Transfer the 5-mL aliquot back to the beaker and continue to add Phenol Solution until no turbidity is formed in subsequent 5-mL aliquots.
22. Transfer the entire sample into a 500-mL volumetric flask. Rinse the beaker with deionized water and add to the flask.
23. Dilute to volume with deionized water, stopper and mix well. The distillate is now ready for analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *3.2.2 Standard Additions* for more information.
4. Snap the neck off a Selenium 2-mL Ampule Standard, 100-mg/L Se.
5. Prepare three sample spikes. Fill three mixing cylinders with 100 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch

View: Fit, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 0.5-mg/L Se standard solution as follows:

1. Pipet 1.00 mL of a Selenium Standard Solution, 100-mg/L, into a 200-mL volumetric flask. Dilute to volume with deionized water. Transfer 100 mL of the standard into a 500-mL Erlenmeyer flask. Perform the test as described above.
2. To adjust the calibration curve using the reading obtained with the 0.5-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.500 mg/L Se

Program	95% Confidence Limits of Distribution
640	0.486–0.514 mg/L Se

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.01 mg/L Se

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

An EDTA masking agent is added to the sample to remove interferences such as iron prior to the test. The addition of a sulfate buffer adjusts the sample to the optimum pH of 1 to 2. Under these conditions, diaminobenzidine reacts with all selenium present as selenite (Se^{4+}) to give a yellow-colored piazselenol complex which is extracted and the color intensity measured colorimetrically. Selenium present as Se^{2+} and Se^{6+} is not detected unless the sample is distilled. Test results are measured at 420 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Selenium Reagent Set (100 tests)			22442-00
Includes:			
(1) Buffer Solution, sulfate type, pH 2.0	10 mL	500 mL	452-49
(1) Diaminobenzidine, tetrahydrochloride	0.1 g	5 g	7062-22
(2) Potassium Hydroxide Standard Solution, 12 N	4 mL	100 mL	230-32
(1) TitraVer® Hardness Reagent, ACS	0.4 g	100 g	204-26
(1) Toluene, ACS	60 mL	4 liters	14470-17
Water, deionized	100 mL	4 liters	272-56

Required Apparatus

Cotton Balls, absorbent	1	100/pkg	2572-01
Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 100-mL	1	each	508-42
Dropper, 0.5 & 1.0 mL marks, glass	1	5/pkg	14197-05
Dropper, 0.5 & 1.0 mL marks, plastic	1	20/pkg	21247-20
Flask, Erlenmeyer, 500-mL	2	each	505-49
Funnel, separatory, 250-mL	2	each	520-46
Pipet, volumetric, 5-mL	1	each	14515-37
Pipet Filler, safety bulb	1	each	14651-00
Ring, support, (3-inch) 83-mm	1	each	580-00
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Spoon, measuring, 0.05-g	1	each	492-00
Spoon, measuring, 0.2-g	1	each	638-00
Support, ring stand, (5 x 8 inch) 127 x 203 mm	1	each	563-00
Select one based on available voltage:			
Hot Plate, 4" diameter, 120 VAC	1	each	12067-01
Hot Plate, 4" diameter, 240 VAC	1	each	12067-02

Distillation Reagents

Calcium Chloride Solution	1000 mL	428-53
Hydrochloric Acid Standard Solution, 0.1 N	1000 mL	14812-53
Hydrogen Peroxide, 30%, ACS	473 mL	144-11
Methyl Orange Indicator Solution, (0.50-g/L)	500 mL	148-49
Phenol Solution, 30-g/L	29 mL	2112-20
Potassium Permanganate Standard Solution, 1-g/L	100 mL	14164-42
Sodium Hydroxide Standard Solution, 0.100 N	1000 mL	191-53
Sulfuric Acid Standard Solution, 19.2 N	500 mL	2038-49

Required Standards

Selenium Standard Solution, 1000-mg/L	100 mL	22407-42

Distillation Apparatus

Distillation Apparatus Set, general purpose	each	22653-00
Distillation Apparatus Heater, 115 VAC	each	22744-00
Distillation Apparatus Heater, 230 VAC	each	22744-02

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3/04 1ed

Selenium_None_Other_DAB_Eng_Ody.fm

Method 8185 **Silicomolybdate Method**

Powder Pillows **HR (1.0 to 100.0 mg/L)**

Scope and Application: For water and seawater

Tips and Techniques

• Sample temperature should be 15–25 °C (59–77 °F).

Powder Pillows Method 8185

1. Touch

Hach Programs.

Select program

656 Silica HR.

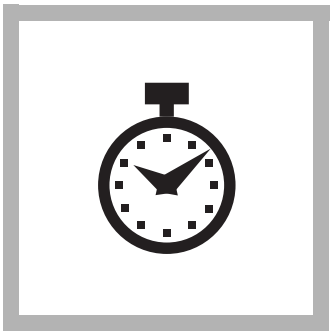
Touch **Start**.

2. Fill a round sample cell with 10-mL of sample.

3. Add the contents of one Molybdate Reagent Powder Pillow for High Range Silica to the sample cell (the prepared sample). Swirl until completely dissolved.

4. Add the contents of one Acid Reagent Powder Pillow for High Range Silica. Swirl to mix.

A yellow color will develop if silica or phosphorus is present.



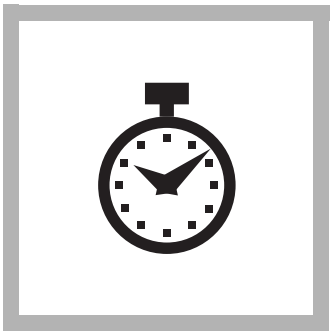
5. Touch the timer icon.

Touch **OK**.

A ten-minute reaction period will begin.

6. When the timer beeps, add the contents of one Citric Acid Powder Pillow to the sample cell. Swirl to mix.

Any yellow color due to phosphorus is removed in this step.



7. Touch the timer icon.

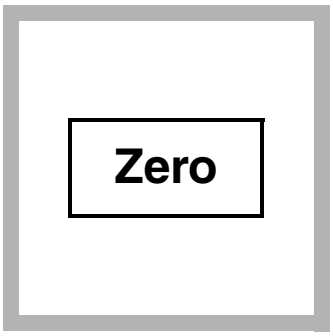
Touch **OK**.

A two-minute reaction period will begin.

Perform *step 8–step 11* within three minutes after the timer beeps.

8. Fill a second sample cell with 10 mL of the original sample (the blank).

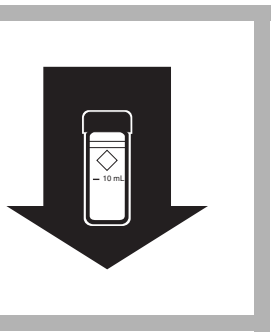
9. Place the blank into the cell holder.



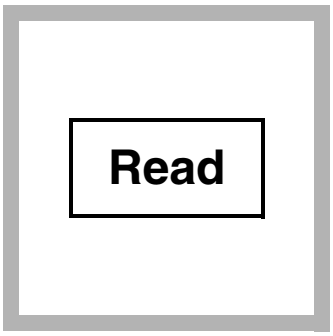
10. Touch **Zero**.

The display will show:

0.0 mg/L SiO_2



11. Place the prepared sample into the cell holder.



12. Touch **Read**.

Results will appear in mg/L SiO_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Color	Eliminated by zeroing the instrument with the original sample.
Iron	High levels of Fe^{2+} and Fe^{3+} interfere.
Phosphate	Does not interfere below 50 mg/L PO_4^{3-}. At 60 mg/L PO_4^{3-}, a negative 2% interference occurs. At 75 mg/L PO_4^{3-}, the interference is negative 11%.
Sulfides (S^{2-})	All levels interfere.
Turbidity	Eliminated by zeroing the instrument with the original sample.

Occasionally a sample contains silica which reacts very slowly with molybdate. The nature of these "molybdate-unreactive" forms is not known. A pretreatment with Sodium Bicarbonate (Cat. No. 776-01), then Sulfuric Acid (Cat. No. 1270-32) will make these forms reactive to molybdate. The pretreatment is given in

Standard Methods for the Examination of Water and Wastewater under Silica-Digestion with Sodium Bicarbonate. A longer reaction time with the sample and the molybdate and acid reagents (before adding citric acid) may help instead of the bicarbonate treatment.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic bottles. Analyze samples as soon as possible after collection. If prompt analysis is not possible, store samples at 4 °C (39 °F) for up to 28 days. Warm samples to room temperature before analyzing.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a 1000 mg/L Silica Standard Solution.
5. Prepare three sample spikes. Fill three sample cells with 10 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. To check test accuracy, use the 50-mg/L Silica Standard Solution. Analyze according to the HR Silica procedure described above using deionized water as the blank.
2. To adjust the calibration curve using the reading obtained with the 50.0-mg/L Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 50.0 mg/L SiO_2

Program	95% Confidence Limits of Distribution
656	43.2–56.8 mg/L SiO_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	1.1 mg/L SiO_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Silica and phosphate in the sample react with molybdate ion under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. Silica is then determined by measuring the remaining yellow color. Test results are measured at 452 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
High Range Silica Reagent Set for 10-mL samples (100 tests)			24296-00
Includes:			
Acid Reagent Powder Pillows for High Range Silica	1	100/pkg	21074-69
Citric Acid Powder Pillows	1	100/pkg	21062-69
Molybdate Reagent Powder Pillows for High Range Silica	1	100/pkg	21073-69
Water, deionized	10 mL	4 liters	272-56

Required Apparatus

Clippers, for opening powder pillows	1	each	968-00
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Silica Standard Solution, 50-mg/L		200 mL	1117-29
Silica Standard Solution, 1000-mg/L		500 mL	194-49

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Method 8186 **Heteropoly Blue Method***

Powder Pillows **LR (0.01 to 1.60 mg/L as SiO_2)**

Scope and Application: For water and seawater

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- The four-minute reaction time in *step 4* is for samples at 20 °C; for samples at 10 °C, wait eight minutes; for samples at 30 °C, wait two minutes.
- The one-minute reaction time in *step 6* is for samples at 20 °C; for samples at 10 °C, wait two minutes; for samples at 30 °C, wait 30 seconds.
- If testing for very low levels of silica, use Method 8282.

Powder Pillows Method 8186

1. Touch

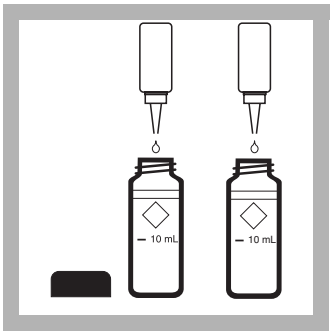
Hach Programs.

Select program

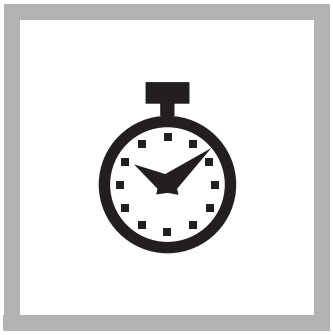
651 Silica LR.

Touch **Start**.

2. Fill two sample cells with 10 mL of sample.



3. Add 14 drops of Molybdate 3 Reagent to each sample cell. Swirl to mix.

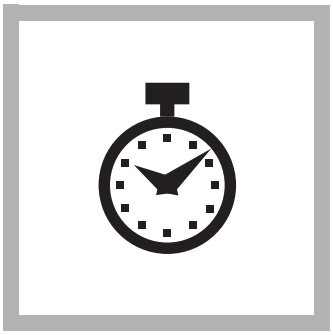


4. Touch the timer icon.

Touch **OK**.

A four-minute reaction period will begin.

5. When the timer beeps, add the contents of one Citric Acid Reagent Powder Pillow to each sample cell. Swirl to mix.



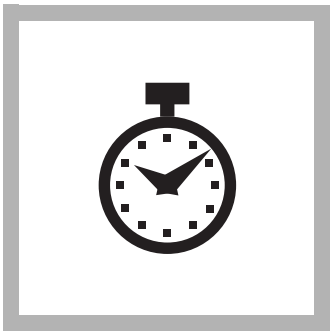
6. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin. The destruction of possible phosphate interference occurs during this period.

7. When the timer beeps, add the contents of one Amino Acid F Reagent Powder Pillow to one of the sample cells. Swirl to mix. This is the prepared sample.

The sample without the Amino Acid F Reagent is the blank.

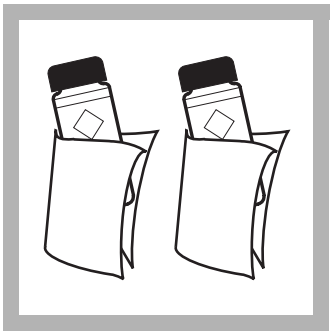


8. Touch the timer icon.

Touch **OK**.

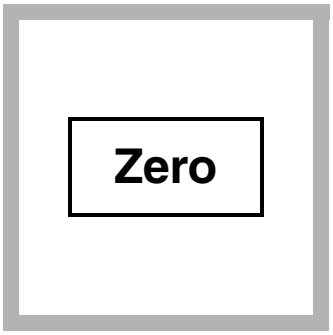
A two-minute reaction period will begin.

A blue color will develop if silica is present.



9. Wipe the cells with a damp cloth, followed by a dry one to remove fingerprints and other marks.

10. When the timer beeps, place the blank into the cell holder.



11. Touch **Zero**.

The display will show:

0.00 mg/L SiO_2

12. Place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L SiO_2.

Interferences

Interfering Substance	Interference Levels and Treatments
Color	Eliminated by zeroing the instrument with the original sample.
Iron	Large amounts interfere.
Phosphate	Does not interfere at levels less than 50 mg/L PO_4. At 60 mg/L PO_4, an interference of –2% occurs. At 75 mg/L PO_4 the interference is -11%.
Slow reacting forms of silica	Occasionally a sample contains silica which reacts very slowly with molybdate. The nature of these "molybdate-unreactive" forms is not known. A pretreatment with Sodium Bicarbonate (Cat. No. 776-01), then Sulfuric Acid (Cat. No. 1270-53) will make these forms reactive to molybdate. The pretreatment is given in *Standard Methods for the Examination of Water and Wastewater* under Silica-Digestion with Sodium Bicarbonate. A longer reaction time with the sample and the molybdate and acid reagents (before adding citric acid) may help instead of the bicarbonate pretreatment.
Sulfides	Interfere at all levels.
Turbidity	Eliminated by zeroing the instrument with the original sample.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic bottles. Analyze samples as soon as possible after collection. If prompt analysis is not possible, store samples for up to 7 days by cooling to 4 °C (39 °F) or below. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Open a 25-mg/L Silica Standard Solution Bottle.
5. Prepare three sample spikes. Fill three sample cells with 10 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use the 1.00-mg/L SiO_2 Standard Solution listed under *Required Standards* in place of the sample. Perform the silica procedure as described above.

2. To adjust the calibration curve using the reading obtained with the 1.00 mg/L Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 1.00 mg/L SiO_2

Program	95% Confidence Limits of Distribution
651	0.95–1.05 mg/L SiO_2

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.013 mg/L SiO_2

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Silica and phosphate in the sample react with molybdate ion under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. An Amino Acid is then added to reduce the yellow silicomolybdic acid to an intense blue color, which is proportional to the silica concentration. Test results are measured at 815 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Low Range Silica Reagent Set (100 tests)			24593-00
Includes:			
(1) Amino Acid F Reagent Powder Pillows (for 10-mL sample)	1 pillow	100/pkg	22540-69
(2) Citric Acid Powder Pillows	2 pillows	100/pkg	21062-69
(2) Molybdate 3 Reagent Solution	1 mL	50 mL	1995-26

Required Apparatus

Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Deionized Water		4 L	272-56
Silica Standard Solution, 1-mg/L SiO_2		500 mL	1106-49
Silica Standard Solution, 25-mg/L as SiO_2		236 mL	21225-31

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On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Method 8282 **Heteropoly Blue Method***

Pour-Thru Cell **ULR (3 to 1000 µg/L as SiO_2)**

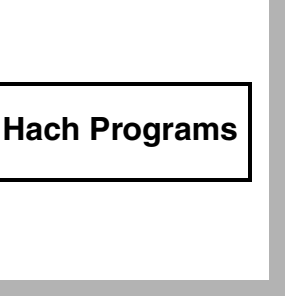
Scope and Application: For testing trace levels of soluble silica in pure and ultrapure water

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

- Reagent blank values printed on analyzer reagent containers vary because the reagents' dilutions vary according to instrument. For this method, use the 1234D analyzer reagent blank value for a 3.78 L volume of Molybdate 3 Reagent (Cat. No. 1995-17). For a Series 5000, 2.9 L volume of Molybdate 3 Reagent (Cat. No. 1995-03), multiply the reagent blank on the label by 1.09. For 100-mL Molybdate 3 Reagent (Cat. No. 1995-32) and 1 L Molybdate 3 Reagent (Cat. No. 1995-53), use the lab blank values on the bottle labels.
- The four-minute reaction time in *step 11* is for samples at 20 °C; for samples at 10 °C, wait eight minutes; for samples at 30 °C, wait two minutes.
- The one-minute reaction time in *step 13* is for samples at 20 °C; for samples at 10 °C, wait two minutes; for samples at 30 °C, wait 30 seconds.
- Clean labware carefully. See *Labware on page 4*.
- Use only plastic containers and labware; glass can contaminate the sample.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

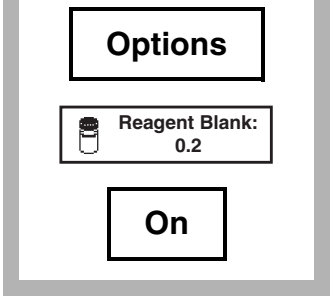
DR/2400 Pour-Thru Cell Method 8282



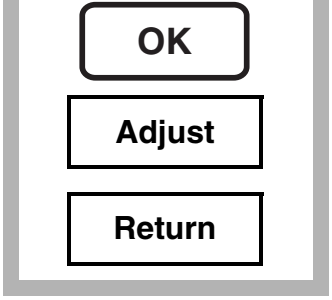
1. Touch **Hach Programs**.

Select program **645 Silica ULR**.

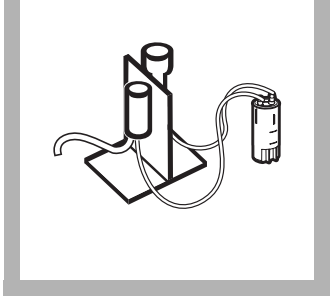
Touch **Start**.



2. Account for the Molybdate 3 reagent blank by touching **Options**, then **Reagent Blank**. Touch **On**.



3. To adjust the reagent blank value, touch the existing value and adjust it with the numeric keypad. Touch **OK**, **Adjust**, and **Return**.



4. Install the Pour-Thru Cell and cell adapter in the sample cell compartment.

Note: *See Section 2.6.1 on page 26 in the Instrument manual for installation details.*

Flush with 50-mL of deionized water.

Note: *Use the 25-mm cell pathlength.*

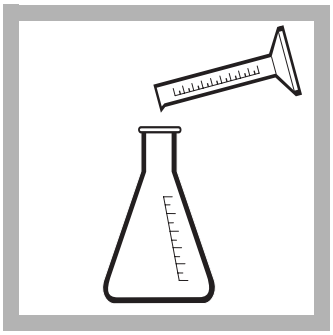
5. Fill two clean 250-mL Erlenmeyer flasks to overflowing with sample.

6. Fill a clean 50-mL plastic graduated cylinder with sample from one of the flasks; then discard the contents of the cylinder.

Repeat three times.

7. Fill the rinsed cylinder to the 50-mL mark with sample from the same flask.

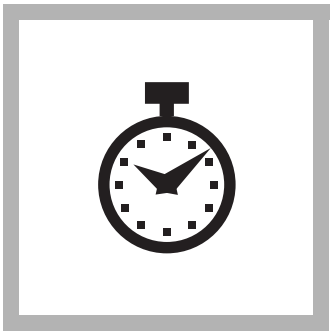
Discard any remaining sample in the flask.



8. Pour the contents of the 50-mL cylinder back into the original flask.

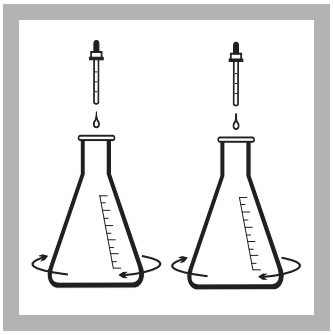
9. Repeat *step 6* through *step 8* for the second flask containing sample.

10. Use a TenSette® Pipet to add 1.0 mL of Molybdate 3 Reagent to each flask. Swirl to mix.

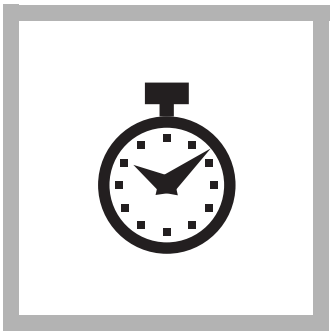


11. Touch the timer icon. Touch **OK**.

A four-minute reaction period will begin.

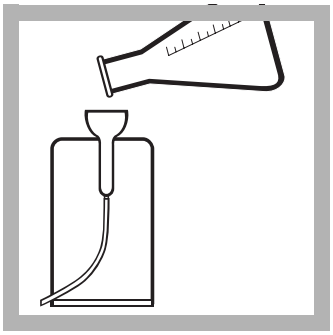


12. When the timer beeps, add 1.0 mL of Citric Acid F Reagent to each flask. Swirl to mix.

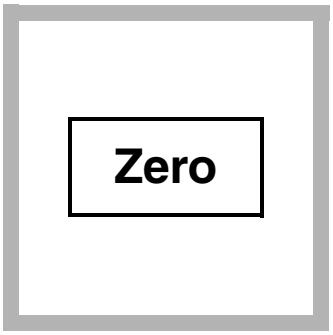


13. Touch the timer icon. Touch **OK**.

A one-minute reaction period will begin. The destruction of possible phosphate interference occurs during this period.



14. When the timer beeps, pour the contents of one flask into the Pour-Thru Cell.



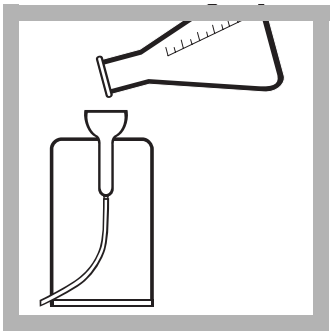
15. After the flow stops, touch **Zero**.

The display will show:

0 µg/L SiO_2

16. Add 1.0 mL of Amino Acid F Reagent to the remaining flask. Swirl to mix.

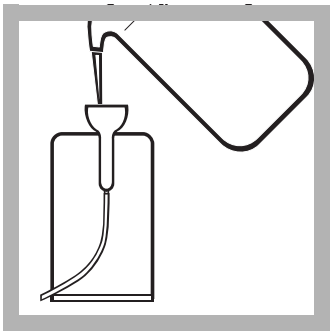
A faint blue color will develop if silica is present.



17. Wait at least 15 seconds, then pour the contents of the second flask into the Pour-Thru Cell.

After the flow stops, touch **Read**.

Results will appear in µg/L SiO_2.



18. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Interfering Substance	Interference Levels and Treatments
Color	Eliminated by zeroing the instrument with the original sample (follow procedure).
Iron	Interferes at high levels.
pH (extreme)	Adjust pH to less than 7. See *Section 3.3 Interferences* on page *41*.
Phosphate (PO_4^{3-})	Interferes at levels greater than 50 mg/L PO_4^{3-}.
Sulfides	Interfere at all levels.
Turbidity	Eliminated by zeroing the instrument with the original sample (follow procedure).

Sample Collection, Storage, and Preservation

Use only plastic containers with tight-fitting closures. Do not use glass containers; they will contaminate the sample with silica. Soak sampling containers with a solution made of one part Molybdate 3 Reagent (Cat. No. 1995-03) to 50 parts of high quality deionized water of low silica concentration. Fill completely and let stand for several hours. Rinse thoroughly with low-level silica water, drain and close. Repeat this cleaning periodically.

Allow the sample stream to flow for 1–2 minutes before collection. Do not adjust the flow during the sampling period as this may introduce particulates. Rinse the container well with sample before collecting the portion for analysis. Analyze as soon as possible.

Reagent Preparation

Amino Acid F Reagent Solution is available in either 100-mL bottles or a package of 20 unit-dose ampules. The bottled reagent is stable for up to one year if the bottle is kept closed when not in use. The ampuled reagent is sealed under argon and is more stable with a shelf life greater than 1 year. Reduced sensitivity at high concentrations (1000 µg/L) indicates reagent instability. Check the bottled reagent on a routine basis by performing an analysis on a 1 mg/L Silica Standard Solution (Cat. No. 1106-49). If the concentration is less than 950 µg/L, use a fresh bottle of Amino Acid F Reagent Solution.

Prepare larger or smaller volumes of Amino Acid F Reagent by dissolving Amino Acid F Reagent Powder in Amino Acid F Reagent Solvent at a ratio of 11 grams per 100 mL. These reagents are available as the Amino Acid F Reagent Package. This prepared solution has limited stability; test routinely with the 1-mg/L Silica Standard Solution as above.

If running a large number of samples, the variable-volume dispensers are convenient. The dispensers are made of fluoropolymer plastic. Do not use a dispenser with glass bottles or glass parts because it will contaminate the reagent with silica.

Labware

All containers used in this test must be cleansed thoroughly to remove any traces of silica. Use plastic containers for all analysis and storage because glass can contaminate the sample with silica. Small bottles or flasks with screw-type closures work well.

Clean containers by normal means (do not use phosphate detergents), then rinse with high quality deionized water of low-level silica concentration. Soak for 10 minutes with a 1:50 dilution of Molybdate 3 Reagent in low-level silica water. Rinse repeatedly with either low-level silica water or the sample before use. Keep containers tightly closed when not in use. Fill the Pour-Thru Cell with this same mixture of Molybdate 3 and water, and let stand for several minutes before use. Rinse with low-level silica water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. Remove the color by rinsing with a 1:5 dilution of ammonium hydroxide, followed by several deionized water rinses. Cover the Pour-Thru Cell when it is not in use.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.

3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three samples as instructed in the procedure. Fill three 250-mL Erlenmeyer flasks with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of 1 mg/L (1000 µg/L) Silica standard, respectively, to each flask and mix thoroughly.
5. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use the 500 µg/L SiO_2 Standard Solution listed under Required Standards in place of the sample. Perform the silica procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 500-µg/L Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 500 µg/L silica

Program	95% Confidence Limits of Distribution
645	497.6–502.4 µg/L silica

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	12 µg/L silica

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

A number of modifications are necessary to adapt the Low Range Silica method for analyzing trace levels in the Ultra Low Range method. It is absolutely necessary to use the one-inch Flow-Thru and liquid reagents. The Flow-Thru increases the reproducibility of the optics and reduces the instability of the readings that result from moveable sample cells. Liquid reagents produce more reproducible readings and lower blank values by eliminating slight turbidity that may remain when using powdered reagents. In addition, the liquid reagents are directly used with Hach process analyzers for continuous silica measurement.

Silica and phosphate in the sample react with molybdate ion under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. Amino Acid F Reagent is then added to reduce the yellow silicomolybdic acid to an intense blue color, which is proportional to the silica concentration. Test results are measured at 815 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
ULR Silica Reagent Set (using Amino Acid F solution, 100 tests)			25535-00
Includes: (2) 1995-32, (2) 22542-32, (1) 23864-42			
ULR Silica Reagent Set (using Amino Acid F ampules, 40 tests)			25814-00
Includes: (1) 1995-32, (1) 22542-32, (2) 23864-20			
Amino Acid F Reagent Solution	1.0 mL	100 mL	23864-42
or			
Amino Acid F Reagent Solution, 1.2-mL Ampules	1 each	20/pkg	23864-20
Citric Acid Reagent Solution	2 mL	500 mL	22542-49
Molybdate 3 Reagent Solution	2.0 mL	1 L	1995-53

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cylinder, graduated, 50-mL, poly	1	each	1081-41
Flask, Erlenmeyer, 250-mL, PMP, w/cap	2	each	20898-46
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 Pipet	5	50/pkg	21856-96
Pour-Thru Cell Kit	1	each	59404-00

Required Standards

Silica Standard Solution, 1 mg/L SiO_2	500 mL	1106-49
Silica Standard Solution, 10 mg/L SiO_2	500 mL	1403-49
Silica Standard Solution, 500 µg/L SiO_2	3.78 L	21008-17

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FAX: (970) 669-2932

Method 8282 **Heteropoly Blue Rapid Liquid Method***

Pour-Thru Cell **ULR(3 to 1000 µg/L as SiO_2)**

Scope and Application: For testing trace levels of soluble silica in pure and ultrapure water

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Tips and Techniques

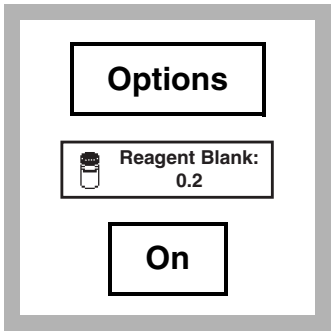
- Reagent blank values printed on analyzer reagent containers vary because the reagents' dilutions vary according to instrument. For this method, use the 1234D analyzer reagent blank value for a 3.78 L volume of Molybdate 3 Reagent (Cat. No. 1995-17). For a Series 5000, 2.9 L volume of Molybdate 3 Reagent (Cat. No. 1995-03), multiply the reagent blank on the label by 1.09. For 100-mL Molybdate 3 Reagent (Cat. No. 1995-32) and 1 L Molybdate 3 Reagent (Cat. No. 1995-53), use the lab blank values on the bottle labels.
- The four-minute reaction time in *step 11* is for samples at 20 °C; for samples at 10 °C, wait eight minutes; for samples at 30 °C, wait two minutes.
- The one-minute reaction time in *step 13* is for samples at 20 °C; for samples at 10 °C, wait two minutes; for samples at 30 °C, wait 30 seconds.
- See *Reagent Preparation on page 4* for instructions on preparing the Amino Acid F Reagent.
- Clean labware carefully. See *Labware on page 4*.
- Install the Pour-Thru Cell into the adapter so that the long pathlength (1-in./25-mm) is parallel to the front of the instrument.

DR/2400 Pour-Thru Cell Method 8282

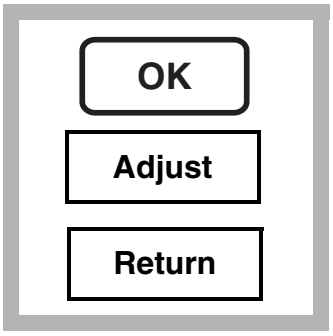
1. Touch **Hach Programs**.

Select program **645 Silica ULR**.

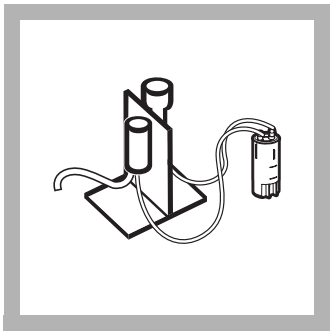
Touch **Start**.



2. Account for the Molybdate 3 reagent blank by touching **Options**, then **Reagent Blank**. Touch **On**.



3. To adjust the reagent blank value, touch the existing value and adjust it with the numeric keypad. Touch **OK**, **Adjust**, and **Return**.



4. Install the Pour-Thru Cell and multi-pathlength cell adapter in the sample cell compartment. Flush with 50-mL of low-silica deionized water.

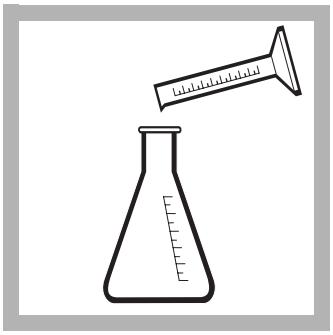
5. Fill two clean 250-mL Erlenmeyer flasks to overflowing with sample.

6. Fill a clean 50-mL plastic graduated cylinder with sample from one of the flasks; then discard the contents.

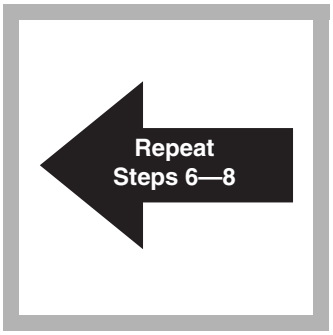
Repeat three times.

7. Fill the rinsed cylinder to the 50-mL mark with sample from the same flask.

Discard any remaining sample in the flask.

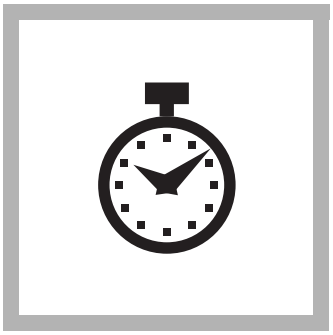


8. Pour the contents of the 50-mL cylinder back into the original flask.



9. Repeat *step 6* through *step 8* for the second flask containing sample.

10. Use the Repipet Jr. dispenser to add 1.0 mL of Molybdate 3 Reagent to each flask. Swirl to mix.

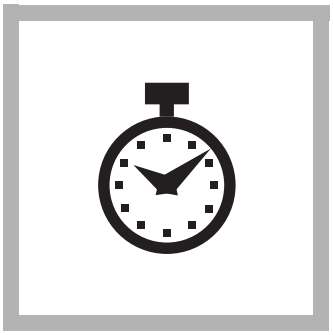


11. Touch the timer icon.

Touch **OK**.

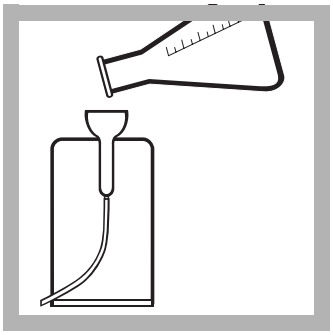
A four-minute reaction period will begin.

12. When the timer beeps, use the Repipet Jr. dispenser to add 1.0 mL of Citric Acid F Reagent to each flask. Swirl to mix.

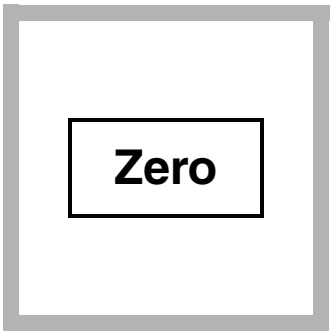


13. Touch the timer icon. Touch **OK**.

A one-minute reaction period will begin. The destruction of possible phosphate interference occurs during this period.



14. When the timer beeps, pour the contents of one flask into the Pour-Thru Cell.



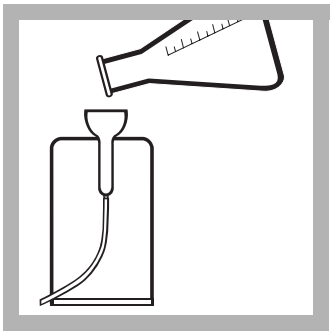
15. After the flow stops, touch **Zero**.

The display will show:

0 µg/L SiO_2

16. Add 1.0 mL of Amino Acid F Reagent to the remaining flask. Swirl to mix.

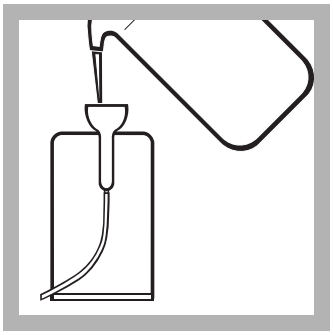
A faint blue color will develop if silica is present.



17. Wait at least 15 seconds, then pour the contents of the second flask into the Pour-Thru Cell.

After the flow stops, touch **Read**.

Results will appear in µg/L SiO_2.



18. Flush the Pour-Thru Cell with at least 50 mL of deionized water immediately after use.

Interferences

Interfering Substance	Interference Levels and Treatments
Color	Eliminated by zeroing the instrument with the original sample (follow procedure).
Iron	Interferes at high levels.
pH (extreme)	Adjust pH to less than 7. See *Section 3.3 Interferences* on page *41.*
Phosphate (PO_4^{3-})	Interferes at levels greater than 50 mg/L PO_4^{3-}.
Sulfides	Interfere at all levels.
Turbidity	Eliminated by zeroing the instrument with the original sample (follow procedure).

Sample Collection, Storage, and Preservation

Use only plastic containers with tight-fitting closures. Do not use glass containers; they will contaminate the sample with silica. Soak sampling containers with a solution made of one part Molybdate 3 Reagent (Cat. No. 1995-03) to 50 parts of high quality deionized water of low silica concentration. Fill completely and let stand for several hours. Rinse thoroughly with low-level silica water, drain and close. Repeat this cleaning periodically.

Allow the sample stream to flow for 1–2 minutes before collection. Do not adjust the flow during the sampling period as this may introduce particulates. Rinse the container well with sample before collecting the portion for analysis. Analyze as soon as possible.

Reagent Preparation

Amino Acid F Reagent Solution is available in either 100-mL bottles or a package of 20 unit-dose ampules. The bottled reagent is stable for up to one year if the bottle is kept closed when not in use. The ampuled reagent is sealed under argon and is more stable with a shelf life greater than 1 year. Reduced sensitivity at high concentrations (1000 µg/L) indicates reagent instability. Check the bottled reagent on a routine basis by performing an analysis on a 1-mg/L Silica Standard Solution (Cat. No. 1106-49). If the concentration is less than 950 µg/L, use a fresh bottle of Amino Acid F Reagent Solution.

Prepare larger or smaller volumes of Amino Acid F Reagent by dissolving Amino Acid F Reagent Powder in Amino Acid F Reagent Solvent at a ratio of 11 grams per 100 mL of reagent solvent. These reagents are available as the Amino Acid F Reagent Package. This prepared solution has limited stability; test routinely with the 1-mg/L Silica Standard Solution.

Labware

All containers used in this test must be cleansed thoroughly to remove any traces of silica. Use plastic containers for all analysis and storage because glass can contaminate the sample with silica. Small bottles or flasks with screw-type closures work well.

Clean containers by normal means (do not use phosphate detergents), then rinse with high quality deionized water of low-level silica concentration. Soak for 10 minutes with a 1:50 dilution of Molybdate 3 Reagent in low-level silica water. Rinse repeatedly with either low-level silica water or the sample before use. Keep containers tightly closed when not in use. Fill the Pour-Thru Cell with this same mixture of Molybdate 3 and water, and let stand for several minutes before use. Rinse with low-level silica water.

Cleaning the Pour-Thru Cell

The Pour-Thru Cell may accumulate a buildup of colored products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurement. Remove the color by rinsing with a 1:5 dilution of ammonium hydroxide, followed by several deionized water rinses. Cover the Pour-Thru Cell when it is not in use.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three samples as instructed in the procedure. Fill three 250-mL Erlenmeyer flasks with 50 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of 1-mg/L (1000-µg/L) Silica standard, respectively, to each flask and mix thoroughly.
5. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Use the 500-µg/L SiO_2 Standard Solution listed under Required Standards in place of the sample. Perform the silica procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 500-µg/L Standard Solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

For more information, see *Section 3.2.4 Adjusting the Standard Curve* on page *40*.

Method Performance

Precision

Standard: 500 µg/L silica

Program	95% Confidence Limits of Distribution
645	497.6–502.4 µg/L silica

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	12 µg/L silica

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

A number of modifications are necessary to adapt the Low Range Silica method for analyzing trace levels in the Ultra Low Range method. It is absolutely necessary to use the one-inch Flow-Thru Cell and liquid reagents. The Flow-Thru Cell increases the reproducibility of the optics and reduces the instability of the readings that result from moveable sample cells. Liquid reagents produce more reproducible readings and lower blank values by eliminating slight turbidity that may remain when using powdered reagents. In addition, the liquid reagents are directly used with Hach process analyzers for continuous silica measurement.

Silica and phosphate in the sample react with molybdate ions under acidic conditions to form yellow silicomolybdic acid complexes and phosphomolybdic acid complexes. Addition of citric acid destroys the phosphate complexes. Amino Acid F Reagent is then added to reduce the yellow silicomolybdic acid to an intense blue color, which is proportional to the silica concentration. Test results are measured at 815 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Rapid Liquid ULR Silica Reagent Set			26785-00
Includes:			
Amino Acid F Reagent Powder		55 g	26511-55
Amino Acid Reagent Dilution Solvent		475 mL	23530-11
Citric Acid F Reagent	1 mL	500 mL	22542-49
Molybdate 3 Reagent	1 mL	500 mL	1995-49

Required Apparatus

Adapter, multi-pathlength cell	1	each	59466-00
Cylinder, graduated, 50-mL, poly	1	each	1081-41
Dispenser, fixed volume, 1.0-mL, w/bottle	3	each	21113-02
Flask, Erlenmeyer, 250-mL, PMP, w/cap	2	each	20898-46
Pour-Thru Cell Kit	1	each	59404-00

Required Standards

Silica Standard Solution, 1-mg/L SiO_2	500 mL	1106-49
Silica Standard Solution, 10-mg/L SiO_2	500 mL	1403-49
Silica Standard Solution, 500-µg/L SiO_2	3.78 L	21008-17
Water, deionized	4 L	272-56

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

Silver

Method 8120

Powder Pillows

Colorimetric Method

(0.005 to 0.700 mg/L)

Scope and Application: For water and wastewater.

Tips and Techniques

- Digestion is required in samples with interferences. See *Digestion on page 4*.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- The graduated cylinder must be completely dry before beginning the test. If the Silver 1 Powder becomes moist, it will not dissolve completely, which will inhibit color development.
- The sample pH for this test must be between 9 and 10. Do not use a pH meter to adjust the sample pH as it will cause contamination. See *Digestion on page 4* for the procedure to adjust pH.
- Generate a blank for each sample.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

Powder Pillows — Method 8120

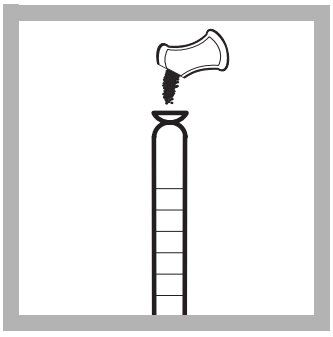
1. Touch

Hach Programs.

Select program

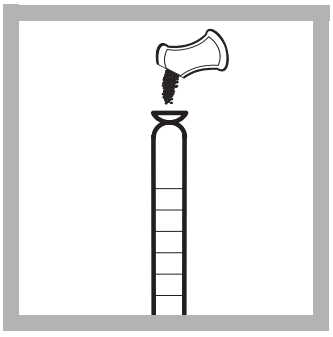
660 Silver.

Touch **Start**.



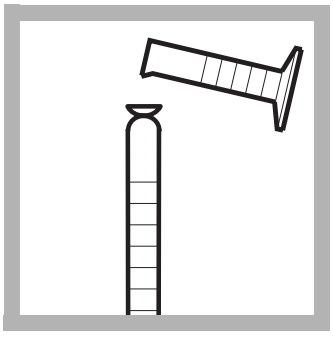
2. Add the contents of one Silver 1 Powder Pillow to a dry 50-mL graduated mixing cylinder.

If the Silver 1 Powder becomes wet at this point, the powder will not dissolve completely, which will inhibit color development.

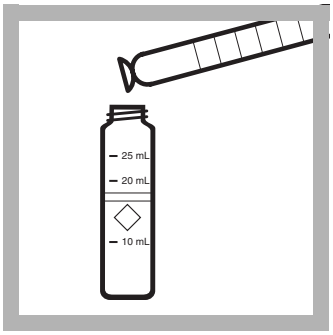


3. Add the contents of one Silver 2 Reagent Solution Pillow to the cylinder. Swirl to completely wet the powder.

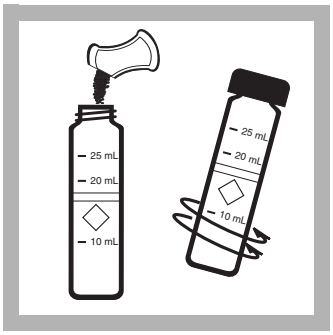
If clumps of dry powder are present when the sample is poured in, the powder will not dissolve completely, which will inhibit color development.



4. Use a 50-mL graduated cylinder to add 50 mL of sample to the 50-mL graduated mixing cylinder. Stopper and invert repeatedly for one minute.



5. Fill a cell to the 25-mL mark with the mixture. (This is the blank.)

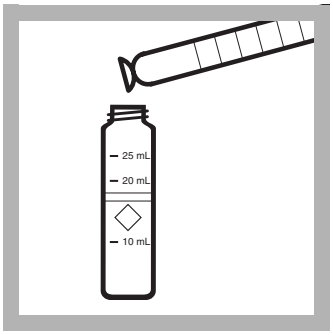


6. Add the contents of one Sodium Thiosulfate Powder Pillow to the blank. Swirl for 30 seconds to mix.

7. Touch the timer icon.

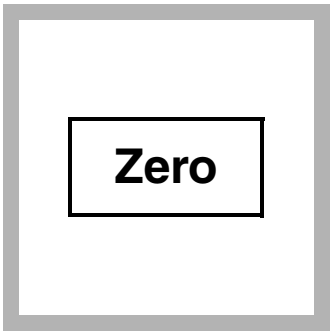
Touch **OK**.

A two-minute reaction period will begin.



8. Fill a second cell to the 25-mL mark from the remaining portion of the mixture. (This is the prepared sample.)

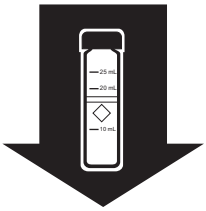
9. When the timer beeps, place the blank into the cell holder.



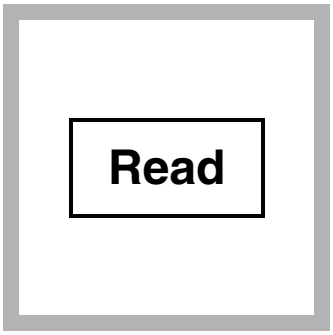
10. Touch **Zero**.

The display will show:

0.000 mg/L Ag



11. Place the prepared sample into the cell holder.



12. Touch **Read**.

Results will appear in mg/L Ag.

Interferences

Interference studies were conducted by preparing a known silver solution (about 0.4 mg/L) and the potential interfering ion. The ion was said to interfere when the resulting concentration changed by ±10%.

Interfering Substance	Interference Levels and Treatments
Aluminum	Negative interference above 30 mg/L
Ammonia	Negative interference above 750 mg/L
Cadmium	Negative interference above 15 mg/L
Calcium	Positive interference above 600 mg/L
Chloride	Negative interference above 19 mg/
Chromium^{6+}	Negative interference above 90 mg/L
Copper	Negative interference above 7 mg/L
Iron	Negative interference above 30 mg/L
Lead	Negative interference above 13 mg/L

(continued)

Interfering Substance	Interference Levels and Treatments
Manganese	Negative interference above 19 mg/L
Magnesium	Positive interference above 2000 mg/L
Mercury	Positive interference above 2 mg/L
Nickel	Negative interference above 19 mg/L
Zinc	Negative interference above 70 mg/L

Sample Collection, Storage, and Preservation

Collect samples in acid-cleaned glass or plastic bottles. Using pH paper, adjust the pH to 2 or less with Concentrated Nitric Acid (Cat. No. 152-49) (about 2 mL/liter). Store preserved samples at room temperature for up to 6 months. If the sample contains particulates or only dissolved metal content is being determined, filter through a 0.45 mm filter at collection. After filtration, adjust the pH to 2 or less as described above.

Before analysis, adjust the pH to 9–10 with 5.0 N Sodium Hydroxide (Cat. No. 2450-32). (See *step 13–step 14* of the Digestion procedure on *page 4*.) Do not use a pH meter because of silver contamination from the electrode. Correct for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Add 5.00 mL of 1000 mg/L Silver Standard Solution to a 100-mL volumetric Class A flask. Dilute to volume with deionized water. This is a 50.0 mg/L standard solution.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-41) with 50-mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 0.5 mg/L silver standard solution as follows:

1. Pipet 0.50 mL of Silver Standard Solution, 1000 mg/L, into a 1000-mL volumetric flask using a Class A volumetric pipet. Dilute to the mark with deionized water. Prepare this solution daily. Perform the silver procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 0.5-mg/L silver standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **OK** to accept the displayed concentration. If an alternate concentration is used, touch the number in the **Adjust to** field and enter the actual concentration. Touch **OK**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 0.500 mg/L Ag

Program	95% Confidence Limits of Distribution
660	0.493–0.507 mg/L Ag

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.005 mg/L Ag

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Digestion

This digestion is for samples containing organic matter, thiosulfate or cyanide. Possible sources for these compounds are wastewater, silver electroplating baths and silver strike solutions. Digestion should be done with a Digesdahl Digestion Apparatus.

Warning: Always wear safety glasses and use a safety shield, or operate the Digesdahl within a closed fume hood. Follow the additional safety precautions in the Digesdahl Digestion Apparatus Manual.

Caution: Poisonous hydrogen cyanide gas may be generated during this digestion. Use a fume hood.

1. Add an appropriate size sample to the 100-mL digestion flask for use with the Digesdahl. Add several boiling chips to prevent bumping.

Note: *Appropriate sample size is determined experimentally. The final sample concentration (after dilution to 100 mL) should be 0–0.6 mg/L. Larger dilutions may be necessary for electroplating baths and silver strike solutions. Do not exceed the maximum sample volume of 25 mL. Several 25-mL aliquots may be digested in succession to concentrate a very dilute sample.*

2. Turn on the water aspirator and make sure there is suction in the fractionating head.

3. Add 3 mL of concentrated sulfuric acid to the sample in the volumetric flask. Immediately place the head on the digestion flask. Never use less than 3 mL of acid.
4. Place the digestion flask on the heater. Turn the temperature dial to 440 °C (825 °F).
5. After the sample begins to char or the sulfuric acid reflux line becomes visible, wait 3–5 minutes.
6. Visually confirm the presence of acid in the flask before adding hydrogen peroxide!
7. Add 10 mL of 50% hydrogen peroxide to the sample via the capillary funnel in the fractionating head.
8. After the hydrogen peroxide has boiled off, heat the sample until heavy white sulfuric acid fumes are present. Continue heating and reduce the sample volume to near dryness. Do not let the sample go completely dry at any time.

Note: *If the sample goes to dryness, turn the Digesdahl off and cool completely. Add water to flask before handling. Repeat digestion from the beginning.*

Note: *If only thiosulfate is present in the sample, proceed to step 1 of the Colorimetric procedure.*

9. Add another 3 mL of sulfuric acid via the capillary funnel.
10. Add another 5 mL of hydrogen peroxide. Check the solution for digestion completion. If digestion is not complete, continue adding hydrogen peroxide in 5 to 10 mL portions. Several portions may be necessary.

Note: *Digestion is complete when the digestate is colorless or the color of the digestate does not change upon addition of hydrogen peroxide. Also, a completely digested sample will not foam.*

11. After digestion is complete and all the hydrogen peroxide is boiled off, reduce the volume of the digestate to near dryness. Do not allow the sample to become completely dry. Remove the flask from the heater. Cool to room temperature.
12. Slowly add about 25 mL of deionized water to the cooled flask.
13. Add 2 drops of 1 g/L Phenolphthalein Indicator Solution. Add 2 drops of 1 g/L Thymolphthalein Indicator Solution.
14. Using sodium hydroxide, adjust the pH of the solution to 9–10. The solution will be pink in this pH range.

Note: *A purple color indicates a pH greater than 10. If this occurs, add a drop of sulfuric acid and 2 drops of each indicator; repeat pH adjustment. Initially, use 50% sodium hydroxide, then 1 N sodium hydroxide as the end point is approached.*

15. Filter turbid digestates. Quantitatively transfer the filtrate (or unfiltered sample) to a clean 100-mL volumetric flask. Dilute to the mark with deionized water. The sample is ready for analysis.

Summary of Method

Silver ions in basic solution react with cadion 2B to form a green to brown to red-purple complex. The sodium thiosulfate acts as a decolorizing agent for the blank. The Silver 1 and Silver 2 reagents contain the buffer, indicator, and masking agents. Organic extractions are not necessary and this method does not have as many interferences as the traditional dithizone method. Test results are measured at 560 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Silver Reagent Set (50 tests)			22966-00
Includes:			
Silver 1 Reagent Powder Pillow	1 pillow	50/pkg	22935-66
Silver 2 Reagent Solution Pillow	1 pillow	50/pkg	22936-66
Sodium Thiosulfate Powder Pillow	1 pillow	50/pkg	22937-66

Required Apparatus

Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 50-mL	1	each	21179-41
Cylinder, graduated, mixing, 50-mL	1	each	1896-41
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Digestion Reagents

Hydrogen Peroxide, 50%		490 mL	21196-49
Phenolphthalein Indicator Solution, 1 g/L		15 mL SCDB	1897-36
Sodium Hydroxide Solution, 50%		500 mL	2180-49
Sodium Hydroxide Solution, 1.00 N		100 mL MDB	1045-32
Sulfuric Acid, ACS, concentrated		2.5 L	979-09
Thymolphthalein Indicator Solution, 1 g/L		15 mL SCDB	21853-36
Water, deionized		4 liters	272-56

Required Standards

Silver Standard Solution, 1000 mg/L Ag		100 mL	14613-42
Water, deionized		4 liters	272-56

Digestion Apparatus

Boiling Chips, silicon carbide		500 g	20557-34
Digesdahl Digestion Apparatus, 115 V ac, 50/60 Hz		each	23130-20
Digesdahl Digestion Apparatus, 230 V ac, 50/60 Hz		each	23130-21
Safety Shield, for Digesdahl		each	50030-00

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★Method 8051 SulfaVer 4 Method*

Powder Pillows or AccuVac® Ampuls (2 to 70 mg/L)

Scope and Application: For water, wastewater, and seawater; USEPA accepted for reporting wastewater analyses

* Adapted from *Standard Methods for the Examination of Water and Wastewater*. Procedure is equivalent to USEPA method 375.4 for wastewater.

Tips and Techniques

- You must adjust the standard curve for each new lot of reagent. See *Standard Solutions* following these steps.
- For best results, perform a new calibration for each lot of reagent. See *Calibration Standard Preparation* following these steps.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.
- Filter highly colored or turbid samples using filter paper (Cat. No. 1894-57) and a funnel (Cat. No. 1083-67). Use this sample in *step 5*.
- After adding reagent to the sample, a white turbidity will form if sulfate is present.
- Undissolved powder that has settled does not affect accuracy.
- SulfaVer® 4 contains barium chloride. The final solution will contain barium chloride (D005) at a concentration regulated as a hazardous waste by the Federal RCRA. See *Section 4* for more information on proper disposal of these materials.

Powder Pillows Method 8051

1. Touch

Hach Programs.

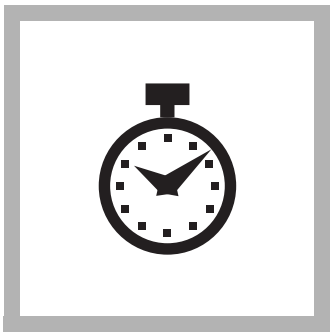
Select program

680 Sulfate.

Touch **Start**.

2. Fill a clean sample cell with 10 mL of sample.

3. Add the contents of one SulfaVer 4 Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.



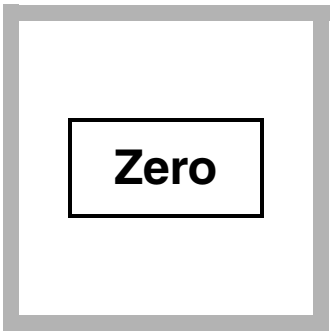
4. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin. Do not disturb the cell during this time.

5. Fill a second sample cell with 10 mL of sample (the blank).

6. When the timer beeps, place the blank into the cell holder.



7. Touch **Zero**.

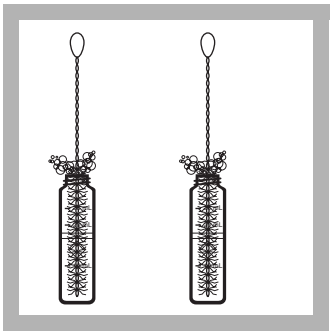
The display will show:

0 mg/L SO_4^{2-}

8. Within five minutes after the timer beeps, place the prepared sample into the cell holder.

Touch **Read**.

Results will appear in mg/L SO_4^{2-}.



9. Clean the sample cells with soap and a brush.

AccuVac Ampul — Method 8051

1. Touch

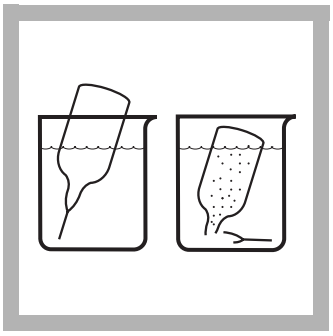
Hach Programs.

Select program

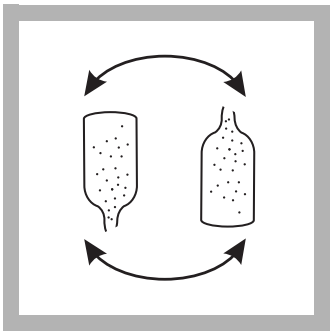
685 Sulfate AV.

Touch **Start**.

2. Fill a clean sample cell with 10 mL of sample (the blank). Collect at least 40 mL of sample in a 50-mL beaker.

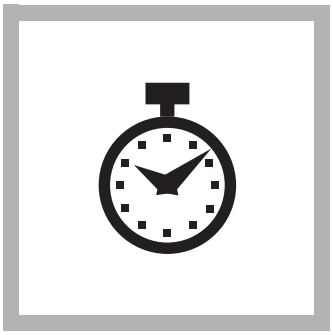


3. Fill a SulfaVer 4 Sulfate AccuVac Ampul with sample. Keep the tip immersed until the ampule fills completely.



4. Quickly invert the ampule several times to mix.

5. Wipe off any liquid or fingerprints from the blank and the ampule.

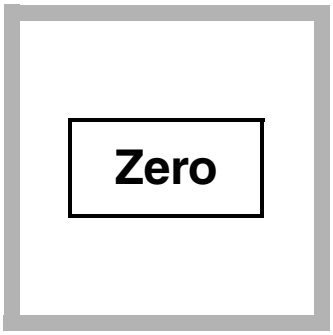


6. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin. Do not disturb the cell during this time.

7. When the timer beeps, place the blank into the cell holder.

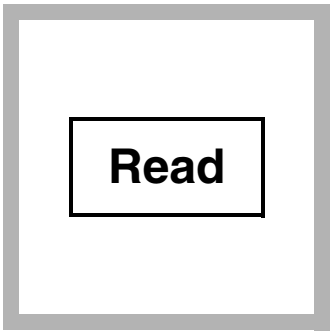


8. Touch **Zero**.

The display will show:

0 mg/L SO_4^{2-}

9. Within five minutes after the timer beeps, place the ampule into the cell holder.



10. Touch **Read**.

Results will appear in mg/L SO_4^{2-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Calcium	Greater than 20,000 mg/L as $CaCO_3$
Chloride	Greater than 40,000 mg/L as Cl
Magnesium	Greater than 10,000 mg/L as $CaCO_3$
Silica	Greater than 500 mg/L as SiO_2

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Samples may be stored up to 7 days by cooling to 4 °C (39 °F) or lower. Warm to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Sulfate 2-mL Ampule Standard, 1000-mg/L sulfate.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Transfer 10 mL of each sample spike to a clean sample cell and analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

Note: *For AccuVac Ampuls, fill three Mixing Cylinders (Cat. No. 1896-41) with 50 mL of sample and spike with 0.2 mL, 0.4 mL, and 0.6 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL Beakers (Cat. No. 500-41). Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by touching* **Read**. *Each addition should reflect approximately 100% recovery.*

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions

Prepare a 70-mg/L sulfate standard solution as follows:

1. Using Class A glassware, Pipet 7 mL of Sulfate Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the SulfaVer procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 70-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **OK** to accept the displayed concentration. If an alternate concentration is used, touch **Adjust** and then enter the actual concentration. Touch **OK**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Calibration Standard Preparation

To perform a sulfate calibration using the SulfaVer method, use Class A glassware to prepare calibration standards containing 10, 20, 30, 40, 50, 60 and 70 mg/L SO_4^{2-} as follows:

1. Into seven different 100-mL Class A volumetric flasks, pipet 1, 2, 3, 4, 5, 6, and 7 mL of the 1000-mg/L Sulfate Standard Solution.
2. Dilute to the mark with deionized water. Mix thoroughly.
3. Using the SulfaVer method and the calibration procedure described in the User-Entered Programs section of the spectrophotometer *Instrument Manual*, generate a calibration curve from the calibration standards prepared above.

Method Performance

Precision
Standard: 30 mg/L SO_4^{2-}

Program	95% Confidence Limits of Distribution
680	27–33 mg/L SO_4^{2-}
685	18–43 mg/L SO_4^{2-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	ΔAbs	ΔConcentration
680	0.010	1 mg/L SO_4^{2-}
685	0.010	2 mg/L SO_4^{2-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Sulfate ions in the sample react with barium in the SulfaVer 4 and form a precipitate of barium sulfate. The amount of turbidity formed is proportional to the sulfate concentration. The SulfaVer 4 also contains a stabilizing agent to hold the precipitate in suspension. Test results are measured at 450 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
SulfaVer® 4 Reagent Powder Pillows	1	100/pkg	21067-69
or			
SulfaVer® 4 Sulfate Reagent AccuVac Ampuls	1	25/pkg	25090-25

Required Apparatus

Beaker, 50-mL	1	each	500-41
Sample cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Sulfate Standard Solution, 1000-mg/L		500 mL	21757-49
Sulfate Standard Solution, 1000-mg/L, 2-mL Ampules		20/pkg	21757-20
Water, deionized		4 liters	272-56

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Sulfate

Turbidimetric Method

(40 to 150 mg/L SO_4^{2-})

UniCell™ Vials

Scope and Application: For wastewater, raw water, and process control

Tips and Techniques

- Adjust the pH of preserved samples to between pH 2–10 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results (See “Accuracy Check” on page 2.).
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure that the temperature of the water sample and the sample vial is between 15–25 °C (59–77 °F).Temperatures outside of this range may give inaccurate results.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

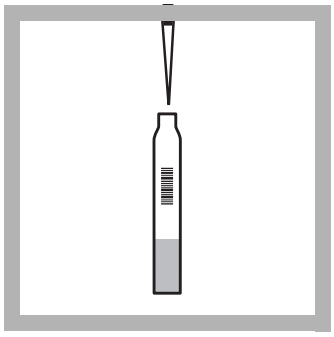
1. Touch

Hach Programs.

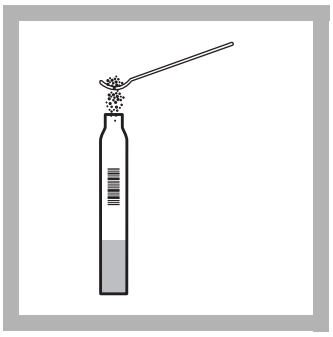
Select program

811 Sulfate, HCT 125.

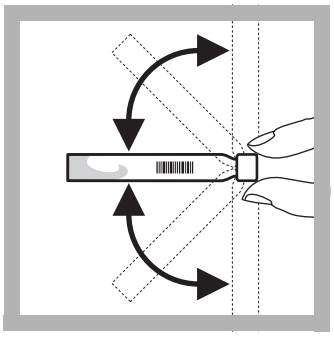
Touch **Start**.



2. Add 5 mL of sample to a sample vial.

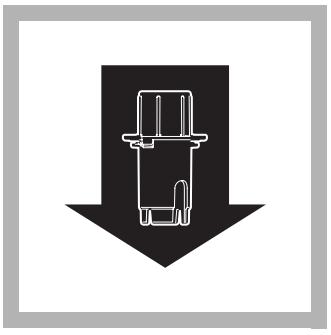


3. Add one spoonful of Barium Chloride A (HCT 125 A) into the sample vial.



4. Immediately cap the sample vial and invert to mix. Mix for 2 minutes.

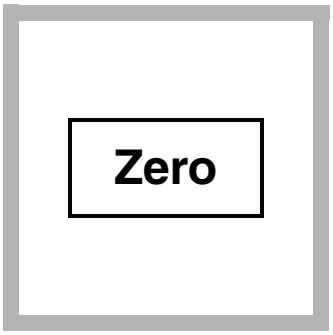
5. Wipe the outside of the zero (white cap) and sample vials with a damp towel, followed by a dry one, to remove fingerprints or other marks.



6. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

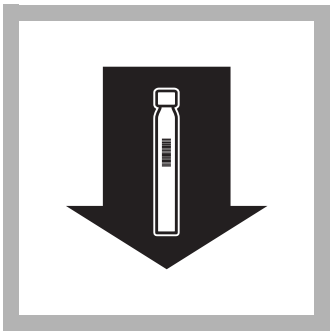
Place the zero vial into the cell adapter.



7. Touch **Zero**.

The display will show:

0 mg/L SO_4^{2-}
Underrange



8. Place the sample in the cell adapter.

Touch **Read**.

Results will appear in mg/L SO_4^{2-}.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	No interference up to:
K^+, Na^+	2000 mg/L
Ca^{2+}, NO_3^-, Cl^-	1000 mg/L
Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, NH_4^+, Ni^{2+}, Si^{2+}, Sn^{2+}, Zn^{2+}	500 mg/L
Al^{3+}, Pb^{2+}, Hg^{2+}, PO_4^{3-}, CO_3^{2-}, I^-, CN^-, NO_2^-	50 mg/L
Cr^{6+}	20 mg/L
Ag^+	2.5 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Samples may be stored up to 28 days at 4 °C (39 °F). Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.

3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row.
4. Prepare three sample spikes. Fill three mixing cylinders with 25 mL of sample. Use a pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of 2500-mg/L SO_4^{2-} standard, respectively, to each sample and mix thoroughly.
5. Transfer 5 mL of each sample spike to a sample vial and analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions

1. To check accuracy, use a 100-mg/L sulfate standard solution listed in *Optional Reagents*. Perform the Sulfate procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 100-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Press **OK** to accept the displayed concentration. If an alternate concentration is used, touch the number in the **Adjust to** field and enter the actual concentration. Touch **OK**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 70 mg/L SO_4^{2-}

Program	95% Confidence Limits of Distribution
811	63–77 mg/L SO_4^{2-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
811	Entire range	0.010	5 mg/L SO_4^{2-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Sulfate ions in the sample react with barium chloride in aqueous solution and form a precipitate of barium sulfate. The resulting turbidity is measured photometrically.

Sulfate

Required Reagents

Description	Unit	Cat. No.
Sulfate - SO_4^{2-}, LR, UniCell™ HCT 125	23/pkg	HCT 125

Optional Reagents

Sulfate Standard, 100-mg/L as SO_4	500 mL	891-49
Sulfate Standard, 2500-mg/L as SO_4	500 mL	14252-49
Sulfate Standard, 2500-mg/L as SO_4, 10-mL Ampules	16/pkg	14252-10

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Cylinder, mixing, graduate 25-mL	each	20886-40
Pipettor, Jencons, 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00
Pipettor, Jencons, 100–1000 µL	each	27949-00
Replacement tips for 27949-00	400/pkg	27950-00

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Sulfate

Turbidimetric Method

(150 to 900 mg/L SO_4^{2-})

UniCell™ Vials

Scope and Application: For wastewater, raw water, and process control

Tips and Techniques

- Adjust the pH of preserved samples to between pH 2–10 before testing.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one.
- Use the standard solution or standard addition method to verify results (See “Accuracy Check” on page 3.).
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- Make sure the temperature of the water sample and the sample vial is between 15–25 °C (59–77 °F). Temperatures outside of this range may give inaccurate results.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

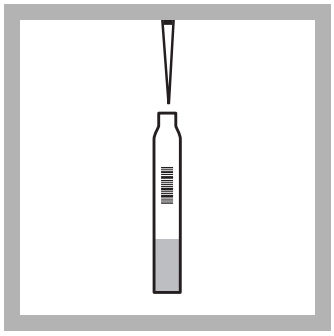
1. Touch

Hach Programs.

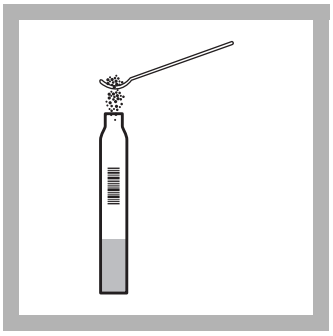
Select program

812 Sulfate, HCT 126.

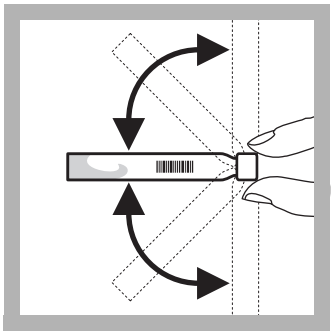
Touch **Start**.



2. Add 2 mL of sample to a sample vial.



3. Add one spoonful of Barium Chloride A (HCT 126 A) into the sample vial.

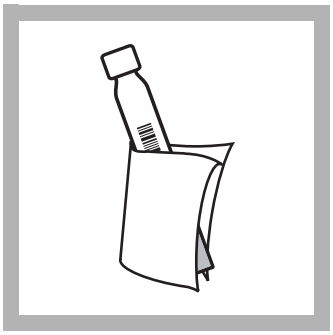


4. Immediately cap the sample vial and invert to mix. Mix for 1 minute.

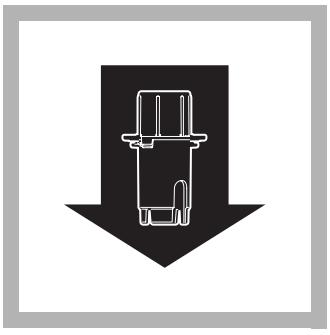
5. Touch the timer icon.

Touch **OK**.

A 30 second reaction period will begin.



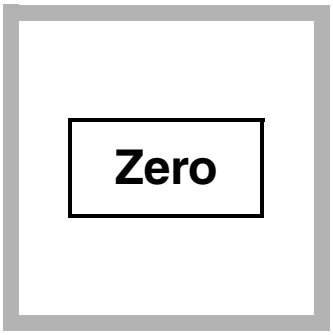
6. Wipe the outside of the zero (**white** cap) and sample vials with a damp towel, followed by a dry one, to remove fingerprints or other marks.



7. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

When the timer beeps, place the zero vial into the cell adapter.



8. Touch **Zero**.

The display will show:

0 mg/L SO_4^{2-}
Underrange

9. Place the sample into the cell adapter.

Touch **Read**.

Results will appear in mg/L SO_4^{2-}.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Interfering Substance	No interference up to:
K^+, Na^+	2000 mg/L
Ca^{2+}, NO_3^-, Cl^-	1000 mg/L
Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, NH_4^+, Ni^{2+}, Si^{2+}, Sn^{2+}, Zn^{2+}	500 mg/L
Al^{3+}, Pb^{2+}, Hg^{2+}, PO_4^{3-}, CO_3^{2-}, I^-, CN^-, NO_2^-	50 mg/L
Cr^{6+}	20 mg/L
Ag^+	2.5 mg/L

Sample Collection, Storage, and Preservation

Analyze samples within 3 hours after collection for best results. Samples may be stored up to 28 days at 4 °C (39 °F). Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row.
4. Prepare three sample spikes. Fill three mixing cylinders with 25 mL of sample. Use a pipet to add 0.2 mL, 0.4 mL, and 0.6 mL of 2500-mg/L SO_4^{2-} standard, respectively, to each sample and mix thoroughly.
5. Transfer 5 mL of each sample spike to a sample vial and analyze each sample spike as described in the procedure above, starting with the 0.2 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions

Prepare a 500-mg/L sulfate standard solution as follows:

1. Using Class A glassware, pipet 20 mL of Sulfate Standard Solution, 2500-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the SulfaVer procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 500-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Press **OK** to accept the displayed concentration. If an alternate concentration is used, touch the number in the **Adjust to** field and enter the actual concentration. Touch **OK**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 750 mg/L SO_4^{2-}

Program	95% Confidence Limits of Distribution
812	672–828 mg/L SO_4^{2-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
812	Entire range	0.010	5 mg/L SO_4^{2-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Sulfate ions in the sample react with barium chloride in aqueous solution and form a precipitate of barium sulfate. The resulting turbidity is measured photometrically.

Required Reagents

Description	Unit	Cat. No.
Sulfate - SO_4^{2-}, HR, UniCell™ HCT 126	23/pkg	HCT 126

Optional Reagents

Sulfate Standard, 2500-mg/L as SO_4	500 mL	14252-49
Sulfate Standard, 2500-mg/L as SO_4, 10-mL Ampules	16/pkg	14252-10

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Apparatus

Cylinder, mixing, graduated 25-mL	each	20886-40
Pipettor, Jencons, 1–5 mL	each	27951-00
Replacement tips for 27951-00	100/pkg	27952-00
Pipettor, Jencons, 100–1000 µL	each	27949-00
Replacement tips for 27949-00	400/pkg	27950-00

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★Method 8131 Methylene Blue Method* (5 to 800 µg/L)

Scope and Application: For testing total sulfides, H_2S, HS^-, and certain metal sulfides in groundwater, wastewater brines, and seawater; USEPA Approved for reporting wastewater analysis**

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Procedure is equivalent to USEPA method 376.2 and Standard Method 4500-S^{2-} D for wastewater.

Tips and Techniques

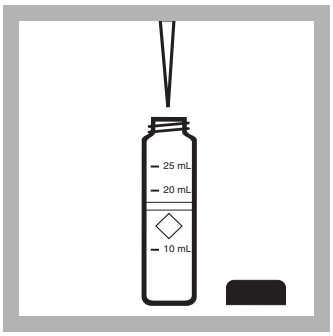
- Analyze samples immediately. Do not preserve for later analysis.
- Avoid excessive agitation of samples to minimize sulfide loss.
- Some sulfide loss may occur if dilution is necessary.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Sulfide 2 reagent contains potassium dichromate. The final solution will contain hexavalent chromium (D007) at a concentration regulated as a hazardous waste by Federal RCRA. Please see *Section 4* for further information on proper disposal of these materials.

Method 8131

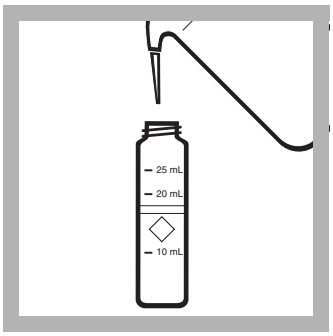
1. Touch **Hach Programs**.

Select program **690 Sulfide**.

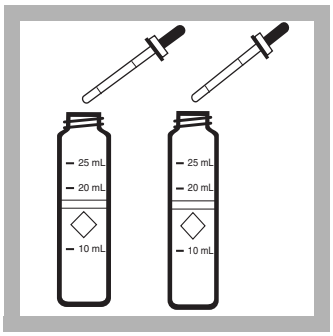
Touch **OK**.



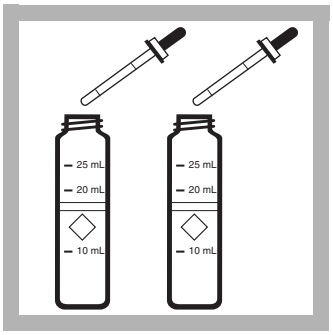
2. Avoiding excess agitation of the sample, use a pipet add 25 mL of sample to a sample cell (the prepared sample).



3. Measure 25 mL of deionized water into a second sample cell (the blank).

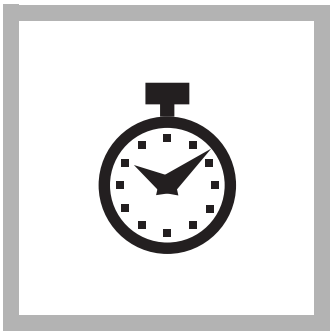


4. Use the calibrated 1-mL dropper to add 1.0 mL of Sulfide 1 Reagent to each cell. Swirl to mix.



5. Use the calibrated 1-mL dropper to add 1.0 mL of Sulfide 2 Reagent to each cell. Cap the cell and immediately invert to mix.

A pink color will develop, then the solution will turn blue if sulfide is present.

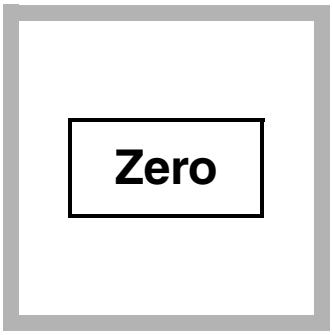


6. Touch the timer icon.

Touch **OK**.

A five-minute reaction period will begin.

7. When the timer beeps, wipe the blank and place it into the cell holder.



8. Touch **Zero**.

The display will show:

0 µg/L S^{2-}

9. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in µg/L S^{2-}.

Determining Soluble Sulfides

Determine soluble sulfides by centrifuging the sample in completely filled, capped tubes and analyzing the supernatant. Insoluble sulfides are then estimated by subtracting the soluble sulfide concentration from the total sulfide result.

Interferences

Interfering Substance	Interference Levels and Treatments
Strong reducing substances (sulfite, thiosulfate and hydrosulfite)	Interfere by reducing the blue color or preventing its development
Sulfide, high levels	High concentrations of sulfide may inhibit full color development and require sample dilution. Some sulfide loss may occur when the sample is diluted.
Turbidity	For turbid samples, prepare a sulfide-free blank as follows. Use it in place of the deionized water blank in the procedure. **1.** Measure 25 mL of sample into a 50-mL Erlenmeyer flask. **2.** Add Bromine Water (Cat. No. 2211-20) dropwise with constant swirling until a permanent yellow color just appears. **3.** Add Phenol Solution (Cat. No. 2112-20) dropwise until the yellow color just disappears. Use this solution to replace the deionized water in *step 3* of the procedure.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation or prolonged exposure to air. Analyze samples immediately.

Method Performance

Precision

Standard: 275 µg/L S^{2-}

Program	95% Confidence Limits of Distribution
690	256–294 µg/L S^{2-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	∆Abs	∆Concentration
Entire range	0.010	5 µg/L S^{2-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Hydrogen sulfide and acid-soluble metal sulfides react with N,N-dimethyl-p-phenylenediamine sulfate to form methylene blue. The intensity of the blue color is proportional to the sulfide concentration. High sulfide levels in oil field waters may be determined after proper dilution. Test results are measured at 665 nm.

Required Reagents

Description	Quantity required per test	Unit	Cat. No.
Sulfide Reagent Set (100 tests)			22445-00
Includes:			
(2) Sulfide 1 Reagent	2 mL	100 mL MDB	1816-32
(2) Sulfide 2 Reagent	2 mL	100 mL MDB	1817-32
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Pipet, volumetric, Class A, 25-mL	1	each	14515-40
Pipet Filler, safety bulb	1	each	14651-00
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

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3/04 1ed

Sulfite

Colorimetric Method*
(0.10 to 5.00 mg/L)

Scope and Application: For boiler water, foodstuffs

* Reagent sets for this method are only available in Europe.

Tips and Techniques

- Samples must be analyzed immediately.
- Sample and Reagent temperature must be between 15–25 °C (59–77 °F).
- Adjust the sample pH between 3–10.

Colorimetric Method

1. Touch

Hach Programs.

Select program

692 Sulfite, HPT 430.

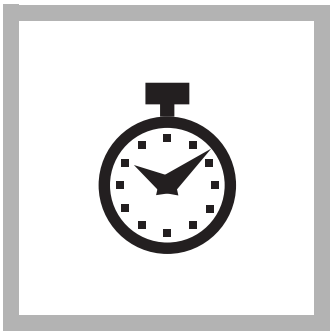
Touch **Start**.

2. Fill a clean sample cell with 10 mL of deionized water. Cap the cell. This is the blank.

3. Pipet 10 mL of sample into a second clean sample cell.

4. Add 5 drops of Sulfite Reagent A (HPT 430 A). Swirl to mix.

5. Add 2 drops of Sulfite Reagent B (HPT 430 B).
Swirl to mix.

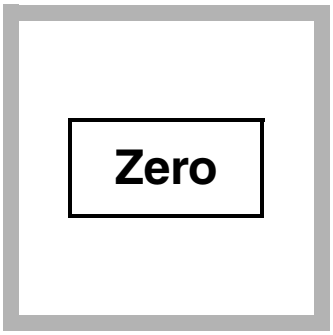


6. Touch the timer icon.

Touch **OK**.

A 3-minute reaction period will begin. Do not disturb the cell during this time.

7. Wipe the blank and place the it into the cell holder.

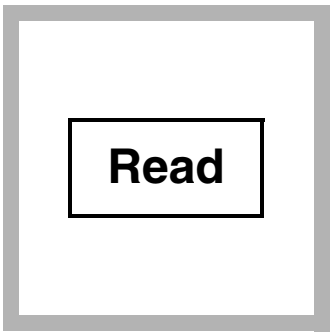


8. Touch **Zero**.

The display will show:

0.00 mg/L SO_3^{2-}

9. When the timer beeps, wipe the prepared sample and place it into the cell holder.



10. Touch **Read**.

Results will appear in mg/L SO_3^{2-}.

Interferences

Interfering Substance	Interference Levels and Treatments
Sulfide	Greater than 5 mg/L

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Samples may be stored up to 7 days by cooling to 4 °C (39 °F) or lower. Warm to 15–25 °C (59–77 °F) before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 20886-40) with 25 mL of sample. Use a pipet to add 1.0 mL, 2.0 mL, and 3.0 mL of 15-mg/L Sulfite Standard Solution, respectively, to each sample and mix thoroughly.
5. Transfer 10 mL of each sample spike to a clean sample cell and analyze each sample spike as described in the procedure above, starting with the 1.0 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
6. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solutions

Prepare a 3.00-mg/L Sulfite standard solution as follows:

1. Using Class A glassware, pipet 20 mL of 15-mg/L Sulfite Standard Solution into a 100-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the Colorimetric Sulfite procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 3.0-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Press **OK** to accept the displayed concentration. If an alternate concentration is used, touch the number in the **Adjust to** field and enter the actual concentration. Touch **OK**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard: 3.00 mg/L SO_3^{2-}

Program	95% Confidence Limits of Distribution
692	2.51–3.49 mg/L SO_3^{2-}

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	ΔAbs	ΔConcentration
692	0.010	0.04 mg/L SO_3^{2-}

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The reagents react with sulfite to form a yellow complex. The samples are measured at 435 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Sulfite Colorimetric Reagent Set*		100/pkg	HPT 430
Includes:			
Sulfite Reagent A	1	28 mL	HPT 430 A
Sulfite Reagent B	1	8.7 mL	HPT 430 B

Required Apparatus

Sample cells, 10-mL, w/cap	2	6/pkg	24276-06

Required Standards

Sulfite Standard Solution, 15-mg/L		500 mL	24084-49
Water, deionized		4 liters	272-56

*Available in Europe only

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DR/2400

Surfactants, Anionic (Detergents)

Method 8028

Crystal Violet Method*

(0.002 to 0.275 mg/L as LAS)

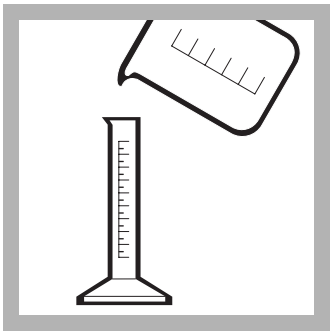
Scope and Application: For water, wastewater, and seawater

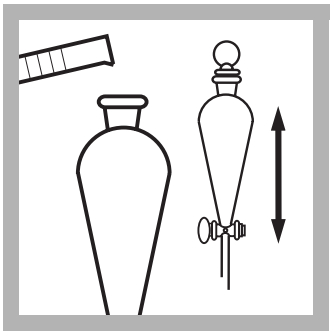
* *Analytical Chemistry,* 38, 791 (1966).

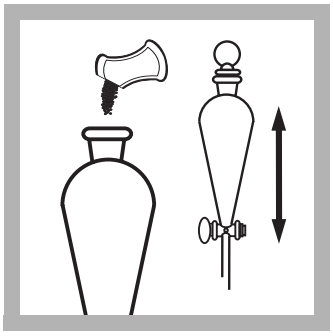
Tips and Techniques

- Use benzene only in a well-ventilated area.
- To prevent water droplets from forming in the sample cells, use only dry sample cells and discard the first few mL of benzene. Additionally, it helps to transfer the liquid from the funnel to a sample cell, let it sit for a few seconds, and decant to a second cell for reading.
- Excessive agitation may cause an emulsion to form, which in turn makes the phases separate more slowly. Should this occur, remove most of the water layer, then gently agitate the contents of the funnel with a clean Teflon®-coated rod or other such inert tool.
- Spilled reagent will affect test accuracy and is hazardous to the skin and other materials.
- Acetone (Cat. No. 14429-49) may be used to clean benzene from glassware.
- Benzene (D018) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Collect water saturated with benzene and benzene solutions for disposal with laboratory solvent wastes. See *Section 4* for more information on proper disposal of these materials.
- For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust. See the instrument manual for more information on *Running a Reagent Blank*.

Method 8028







1. Touch

Hach Programs.

Select program

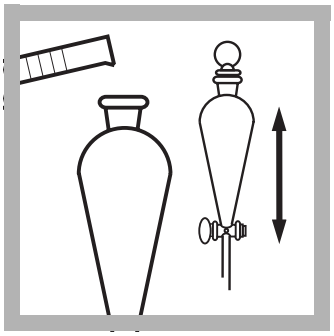
710 Surfactants.

Touch **Start**.

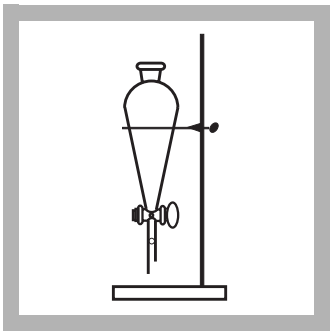
2. Fill a clean 500-mL graduated cylinder to the 300 mL mark with sample. Pour the sample into a clean 500-mL separatory funnel.

3. Add 10 mL of Sulfate Buffer Solution. Stopper the funnel. Shake the funnel for five seconds.

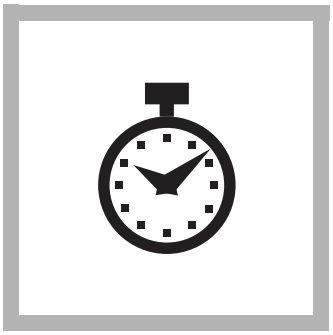
4. Add the contents of one Detergents Reagent Powder Pillow to the funnel. Stopper the funnel and shake until the powder dissolves completely. (This may take some time.)



5. Add 30 mL of benzene to the funnel. Stopper the funnel and shake gently for one minute.



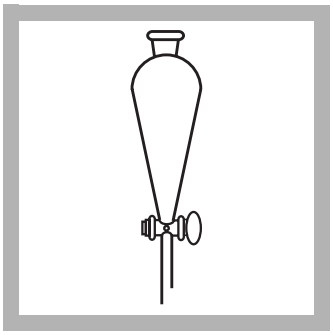
6. Place the separatory funnel in a support stand.



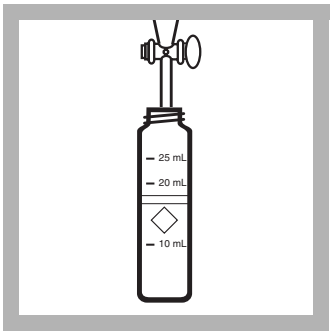
7. Touch the timer icon.

Touch **OK**.

A 30-minute reaction period will begin.

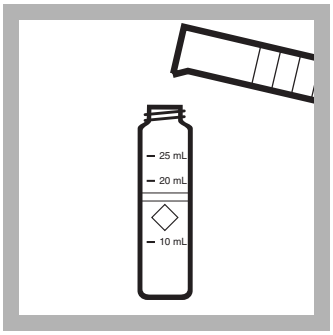


8. After the timer beeps, remove the stopper and drain the bottom water layer. Discard this layer.



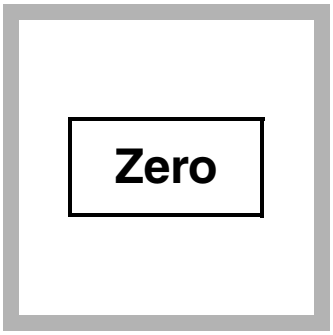
9. Drain the top benzene layer into a clean 25-mL sample cell (this is the prepared sample).

Do not filter the benzene layer before color measurement. Filtration removes the blue color.



10. Fill another sample cell to the 25-mL mark with pure benzene (this is the blank).

11. Place the blank into the cell holder.

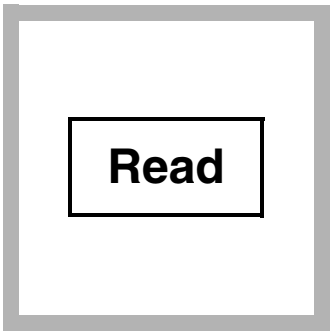


12. Touch **Zero**.

The display will show:

0.000 mg/L LAS

13. Place the prepared sample into the cell holder.



14. Touch Read.

Results will appear in mg/L LAS.

Interferences

Interfering Substance	Interference Levels and Treatments
Chloride	High amounts of chloride, such as those levels found in brines and seawater, will cause low results.
Perchlorate ions	Interferes at all levels.
Periodate ions	Interferes at all levels.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles. Analyze samples as soon as possible, but they may be stored at least 24 hours by cooling to 4 °C (39 °F). Warm to room temperature before testing.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify that the units displayed are in mg/L.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Detergent Voluette® Ampule Standard, 60-mg/L LAS.
5. Prepare three sample spikes. Fill three beakers with 300 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

   Note: *The anionic surfactant reading should increase 0.02 mg/L for every 0.1 mL of standard added.*

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Method Performance

Precision

Standard: 0.180 mg/L LAS

Program	95% Confidence Limits of Distribution
9	0.177–0.183 mg/L LAS

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.002 mg/L LAS

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Detergents, ABS (alkyl benzene sulfonate), or LAS (linear alkylate sulfonate) are determined by association with crystal violet dye and extraction of the ion-pair complex into benzene. Test results are measured at 605 nm.

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Detergents Reagent Set			24468-00
Includes:			
(1) Benzene, ACS	55 mL	4 liters	14440-17
(2) Buffer Solution, sulfate-type	10 mL	500 mL	452-49
(3) Detergent Reagent Powder Pillows	1 pillow	25/pkg	1008-68

Required Apparatus

Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 25-mL	1	each	508-40
Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 500-mL	1	each	508-49
Funnel, separatory, 500-mL	1	each	520-49
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Support Ring, 4-inch	1	each	580-01
Support, Ring Stand, 5 x 8 inch base	1	each	563-00

Required Standards

Detergent Standard Solution, 10-mL Voluette® Ampule, 60-mg/L LAS		16/pkg	14271-10

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FAX: (970) 669-2932

Suspended Solids

Method 8006

Photometric Method*

(0 to 750 mg/L)

Scope and Application: For water and wastewater

* Adapted from *Sewage and Industrial Wastes*, 31, 1159 (1959).

Method 8006

1. Touch

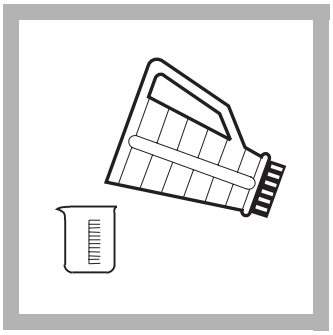
Hach Programs.

Select program

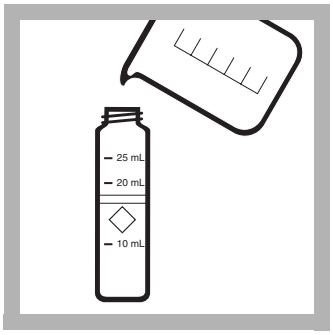
630 Suspended Solids.

Touch **Start**.

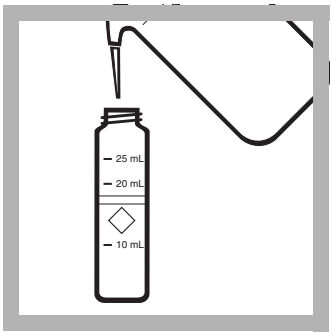
2. Blend 500 mL of sample in a blender at high speed for exactly two minutes.



3. Pour the blended sample into a 600-mL beaker.

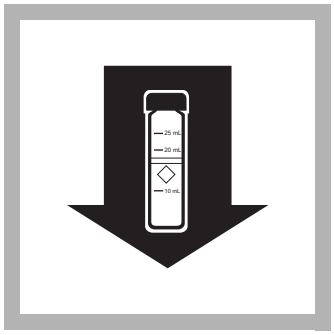


4. Stir the sample and immediately pour 25 mL of the blended sample into a sample cell (the prepared sample).

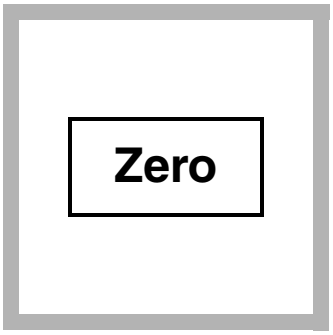


5. Fill a second sample cell with 25 mL of tap water or deionized water (the blank).

Remove gas bubbles in the water by swirling or tapping the bottom of the cell on a table.



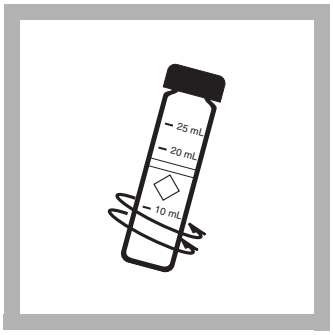
6. Place the blank into the cell holder.



7. Touch **Zero**.

The display will show:

0 mg/L Susp.Solids



8. Swirl the prepared sample to remove any gas bubbles and uniformly suspend any residue.

9. Place the prepared sample into the cell holder.

Interferences

Samples that absorb strongly at 810 nm, such as blue dyes, may give false, high-bias readings. A user-entered calibration is advised for these samples.

Calibration for this test is based on parallel samples using the gravimetric technique on sewage samples from a municipal sewage plant. For most samples, this calibration will provide satisfactory results. When higher accuracy is required, run parallel spectrophotometric and gravimetric determinations with portions of the same sample. The new calibration should be made on your particular sample using a gravimetric technique as a basis.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Analyze samples as soon as possible after collection. The sample may be stored seven days by cooling to 4 °C (39 °F).

Summary of Method

This method of determining suspended solids is a simple, direct measurement which does not require the filtration or ignition/weighing steps that gravimetric procedures do. The USEPA specifies the gravimetric method for solids determinations, while this method is often used for checking in-plant processes. Test results are measured at 810 nm.

Required Apparatus

Description	Quantity Required Per Test	Unit	Cat. No.
Beaker, 600-mL, poly	1	each	1080-52
Blender, 1.2-L, 120 VAC	1	each	26161-00
Blender, 1.2-L, 240 VAC	1	each	26161-02
Cylinder, graduated, 500-mL, poly	1	each	1081-49
Pipet, serologic, 25-mL	1	each	2066-40
Pipet, Filler, safety bulb	1	each	14651-00
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

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Method 8193

Tyrosine Method*
(0.1 to 9.0 mg/L)

Scope and Application: For water, wastewater, and boiler water

* Adapted from Kloster, M.B., *Journal American Water Works Association*, Vol. 66, No. 1, p. 44 (1974)

Tips and Techniques

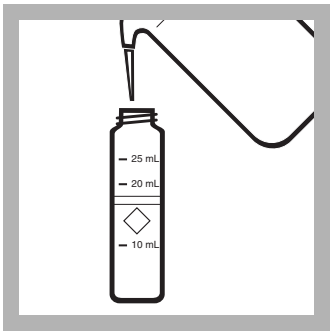
- Filter turbid samples and report results as mg/L soluble tannic acid.
- Results will be given in mg/L tannins (as tannic acid).
- For best accuracy, use a pipet to add the TanniVer® 3 solution.

Method 8193

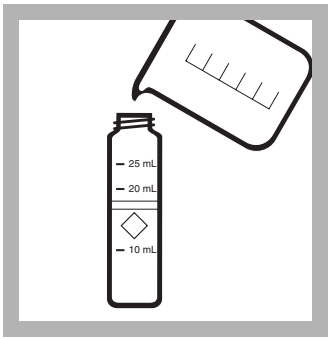
1. Touch **Hach Programs**.

Select program **720 Tannin and Lignin**.

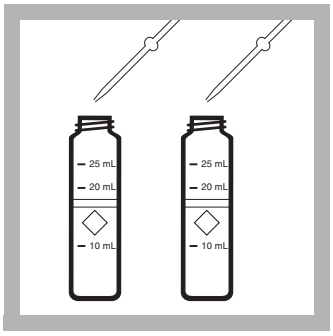
Touch **Start**.



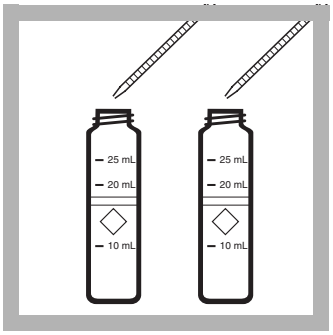
2. Fill a clean sample cell to the 25-mL mark with deionized water (the blank).



3. Fill a clean sample cell to the 25-mL mark with sample (the prepared sample).

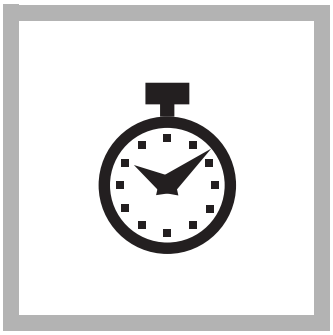


4. Pipet 0.5 mL of TanniVer® 3 Tannin-Lignin Reagent into each cell. Swirl to mix.



5. Pipet 5.0 mL of Sodium Carbonate Solution into each cell. Swirl to mix.

A blue color will develop if tannins and/or lignins are present.

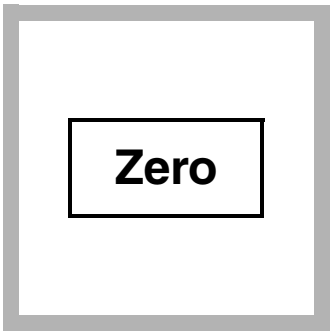


6. Touch the timer icon.

Touch **OK**.

A 25-minute reaction period will begin.

7. When the timer beeps, place the blank into the cell holder.

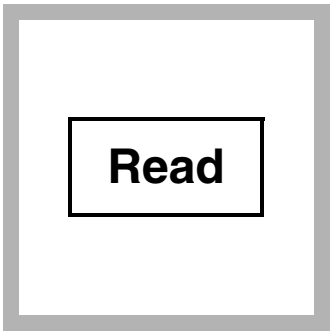


8. Touch **Zero**.

The display will show:

0.0 mg/L Tannins (as Tannic Acid)

9. Place the prepared sample into the cell holder.



10. Touch **Read**.

Results will appear in mg/L Tannins.

Interferences

Interfering Substance	Interference Levels and Treatments
Ferrous iron	Causes a positive interference. (2 mg/L of ferrous iron produces a color equivalent to about 1 mg/L of tannic acid.) To eliminate interference of ferrous iron up to 20 mg/L, add one 0.2 g scoop of Sodium Pyrophosphate (Cat. No. 14295-25) to the sample before testing.
Sulfite	Interference is eliminated by adding 1 mL of formaldehyde (Cat. No. 2059-32) to the sample before testing the sample.

Sample Collection, Storage, and Preservation

Collect samples in clean plastic or glass bottles.

Accuracy Check

Standard Solution Method

Prepare a 200-mg/L tannic acid standard solution as follows:

1. Dissolve 0.200 grams of tannic acid in deionized water and dilute to 1000 mL. Prepare this solution monthly. Further prepare a 6.0-mg/L tannic acid standard by diluting 15.00 mL of the stock solution to 500 mL with deionized water. Prepare this standard daily. Perform the tannin and lignin test on the standard solution as described above.
2. To adjust the calibration curve using the reading obtained with the 6-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration. If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

Method Performance

Precision

Standard: 6.00 mg/L tannic acid

Program	95% Confidence Limits of Distribution
720	5.9–6.1 mg/L tannin

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire range	0.010	0.07 mg/L tannin

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

This test measures all hydroxylated aromatic compounds, including tannin, lignin, phenol, and cresol. This method produces a blue color proportional to the amount of these compounds present in the sample. The results are reported as total tannin and lignin and expressed as mg/L tannic acid. Test results are measured at 700 nm.

Tannin and Lignin

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
Tannin and Lignin Reagent Set (up to 100 tests)			22446-00
Includes:			
(2) Sodium Carbonate Solution	10 mL	500 mL	675-49
(1) TanniVer® 3 Tannin-Lignin Reagent	1 mL	100 mL	2560-32
Water, deionized	25 mL	4 liters	272-56

Required Apparatus

Pipet, Filler, safety bulb	1	each	14651-00
Pipet, volumetric, Class A, 5.0-mL	1	each	14515-37
Pipet, volumetric, Class A, 0.5-mL	1	each	14515-34
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Tannic Acid		113 g	791-14

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TanninLig_None_Other_TYR_Eng_Ody.fm

Method 10017

ToxTrak™ Method * **
(0 to 100% Inhibition)

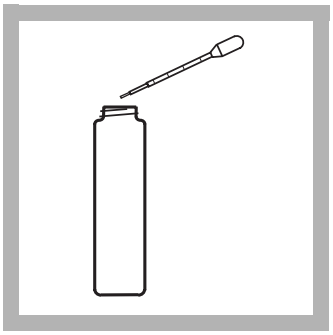
Scope and Application: For water and wastewater

* U.S. Pat. No. 5,413,916
** Liu, D., Bull. Environ. Contm. Toxicol. 26, 145-149 (1981)

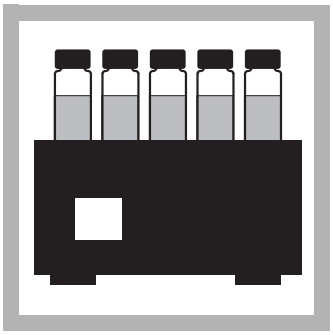
Tips and Techniques

- Do not leave the cells in the instrument during incubation. All samples and control cells should be allowed to react under similar conditions of temperature and light.

Inoculum Development Using Indigenous Biomass

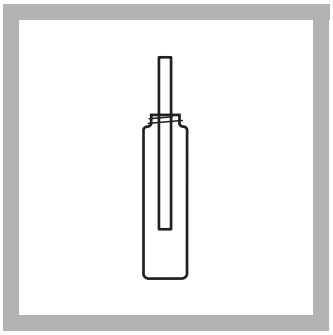


1. Using one of the dropper pipets provided, add 1.0 mL of source culture to a Tryptic Soy Broth Tube.

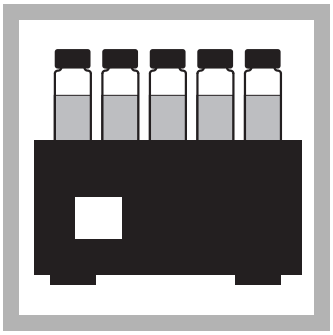


2. Incubate until the vial contents are visibly turbid (turbidity indicates bacterial growth).

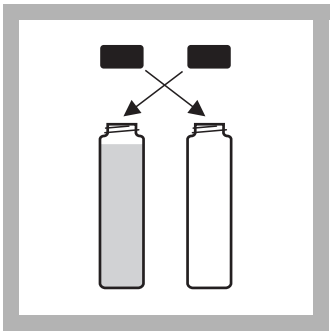
Inoculum Development Using Aqua QC-Stiks



1. Inoculate a Lauryl Tryptose broth tube with an *E. coli* Aqua QC-Stik™ according to the instructions that come with the stick.

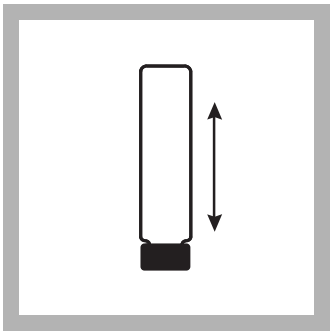


2. Incubate the Lauryl Tryptose Broth Tube at 35 °C (95 °F) until the medium is visibly turbid (approximately 12 hours). Turbidity develops much faster in the incubator than at room temperature.



3. Inoculate a new Lauryl Tryptose Broth Tube by first inverting the tube and switching the caps of the two tubes.

In this way, several medium vials can be inoculated from one Aqua-QC Stick™.

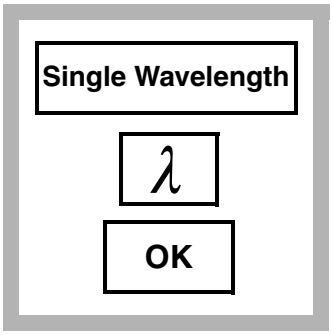


4. Invert the new tube. After incubation, this new vial may be used in subsequent tests.

If toxicity tests will be run on consecutive days, inoculum may be kept several days at room temperature.

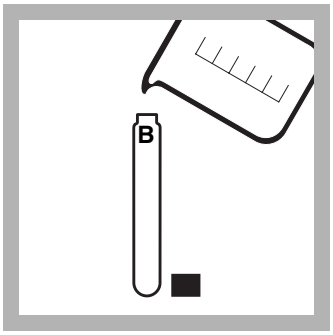
Cultures 10 to 72 hours old give best results.

Test 'N Tube Colorimetric Reaction



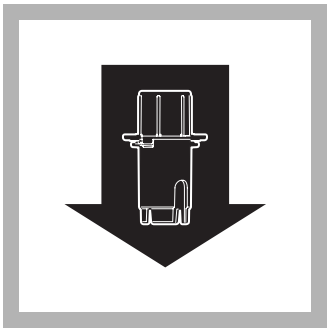
1. Touch **Single Wavelength,** then touch λ.

Enter **603** nm. Touch **OK**.



2. Fill a Test 'N Tube sample cell with deionized water. This is the blank.

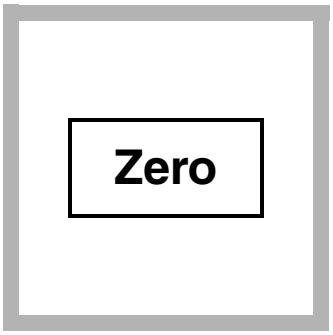
3. Wipe the outside of all the cells with a tissue to remove fingerprints and other marks.



4. Install the 16-mm adapter.

Note: *See Section 2.6 on page 26 in the Instrument Manual for installation details.*

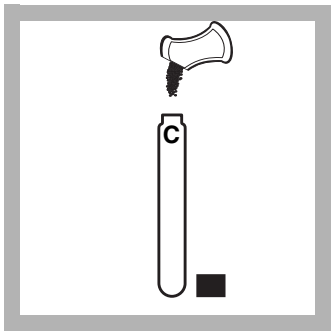
Insert the blank into the adapter.



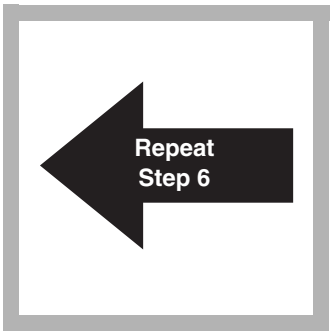
5. Touch **Zero**.

The display will show:

0.000 ABS

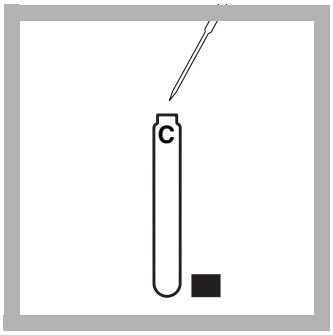


6. Label a cell "control". Open one ToxTrak Reagent Powder Pillow and add the contents to the empty reaction cell.



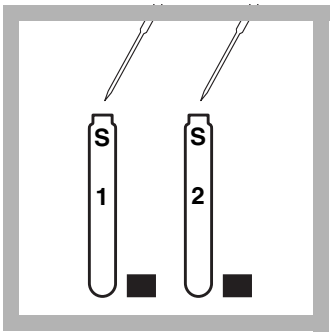
7. For each sample or dilution, repeat *step 6*.

Label each cell.



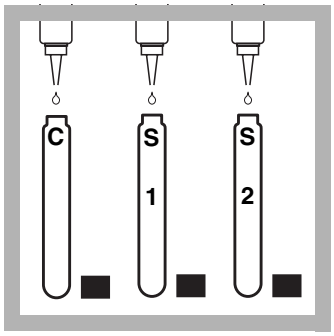
8. Add 5.0 mL of deionized water to the control cell.

Use deionized water that is free of toxicity or another water source that represents baseline toxicity.



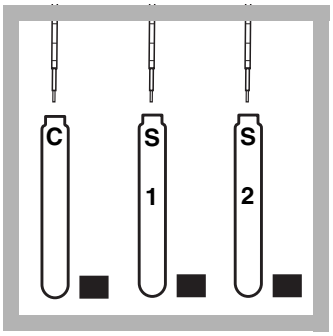
9. Add 5.0 mL of sample (or dilutions) to each sample cell.

To determine the approximate threshold level of toxicity for a sample, dilute 1 mL of sample to 10 mL with deionized water and run the test. Continue to make serial 1/10 dilutions of the sample until a level is reached that gives 0% Inhibition in the final calculation.



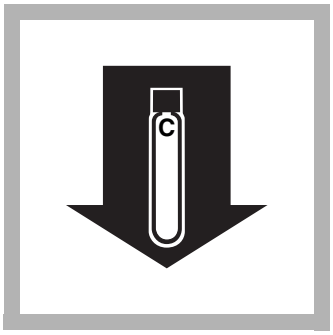
10. Add two drops of Accelerator Solution to each cell. Cap and shake to mix.

Shaking fully oxygenates the samples and assures that the oxygen concentration is not a factor in determining the respiration rate.



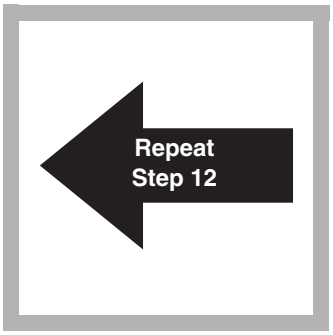
11. Add 0.5 mL of inoculum (previously prepared) to each cell.

Cap and invert to mix.



12. Place the control in the adapter. Record the absorbance.

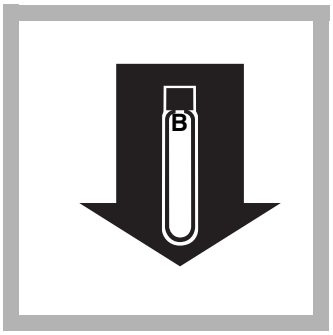
Note: *See the Instrument Manual for more information on taking a reading.*



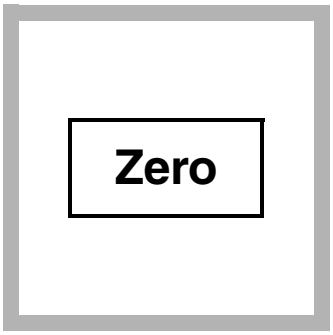
13. Repeat *step 12* for all samples and dilutions. Be sure to record each absorbance.

14. Allow the solutions in the tubes to react until the absorbance of the control decreases 0.60 ±0.10 abs. This takes 45–75 minutes. Invert occasionally.

Reaction time varies according to temperature, age of the culture, bacteria concentrations, etc.



15. After the absorbance of the control has decreased to 0.60 ±0.10 abs., place the blank into the cell adapter.



16. Touch **Zero**.

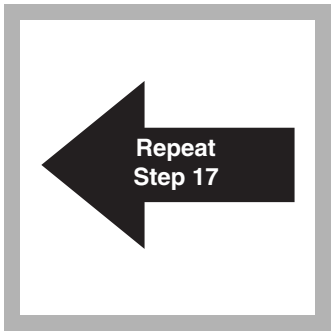
The display will show:

0.000

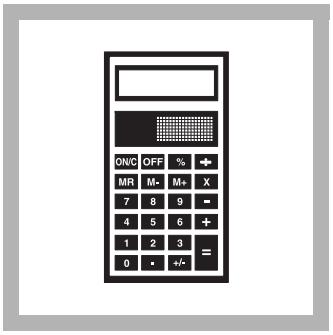
17. Place the control into the cell holder.

Touch **Read**. The absorbance value of the Control will appear.

Record this value.



18. Repeat *step 17* for each sample or dilution. Record each absorbance value.



19. Calculate the % Inhibition:

$$\%I = \left[1 - \left(\frac{\Delta A_{sample}}{\Delta A_{control}}\right)\right] \times 100$$

Where:

ΔA = Initial absorbance value – Final absorbance value

Note: *Some toxins increase respiration and will give a negative % Inhibition on all respiration-based toxicity tests. After repeated testing, samples that give a % Inhibition that is more negative than –10% should be considered toxic.*

Interpreting Results

The % Inhibition results obtained are only a relative measurement. They do not represent a true quantitative measurement of toxic concentration. The % Inhibition does not necessarily increase in direct proportion to the concentration of toxins. To determine the minimum inhibition concentration of a toxin, it is possible to make tenfold dilutions of the sample and determine the % Inhibition for the dilutions until the sample is diluted sufficiently so that no inhibition is observed. This is the No Observed Effect Concentration (NOEC).

Due to the many variables involved in the test, the limits of detection are on the order of 10% Inhibition. This would correlate to the Lowest Observable Effect Concentration (LOEC). If a sample shows less than 10% Inhibition, repeat the test. After several repetitions, look at the series of data to determine the likelihood of toxicity. Results below 10% are not reliable, but can be used to surmise some presence of toxicity if they are consistent. See examples below:

Data Points: % Inhibition	Conclusion
7%, 9%, 5%, 8%, 5%	May be slightly toxic
7%, -4%, -5%, 5%, 1%	Most likely not toxic
-7%, -9%, -5%, -8%, -5%	May be slightly toxic

Some toxins will increase respiration and will give a negative % Inhibition on this and all other respiration-based toxicity tests. After repeated testing, samples that always give a % Inhibition that is more negative than -10% should be considered toxic.

Disposal of Test Cultures

Dispose of active bacterial cultures grown during incubation by using one of these methods:

- Autoclave used test containers at 121 °C (250 °F) for 15 minutes at 15 pounds of pressure. Once the containers are sterile, pour the contents down the drain with running water. The reaction tubes may be washed and re-used.
- Sterilize test containers by using a 1:10 dilution of commercial laundry bleach. Pour the test container contents and test containers into the bleach solution. Allow 10–15 minutes of contact time with the bleach solution. Then pour the liquid down the drain and wash the reaction tubes for reuse.

Summary of Method

This method is based on the reduction of resazurin, a redox-active dye, by bacterial respiration. When it is reduced, resazurin changes color from blue to pink. Toxic substances can inhibit the rate of resazurin reduction. A chemical accelerant has been added to shorten the reaction time.

Toxicity

Required Reagents

Description	Quantity Required per test	Unit	Cat. No.
ToxTrak™ Reagent Set (25 tests)			
(Includes all consumable reagents and apparatus used in the test)			25972-00
Water, deionized	varies	200 mL	272-29
Available Separately:			
Aqua QC-Stiks, *Escherichia coli*	1	3 cultures	27063-03
ToxTrak™ Reagent Powder Pillows	2 pillows	50/pkg	25607-66
ToxTrak™ Accelerator Solution	4 drops	15 mL SCDB	25608-36
Tryptic Soy Broth Tubes	1	15/pkg	22777-00

Required Apparatus

Clippers, to open powder pillows		each	936-00
Dropper, 0.5- and 1.0-mL marks		20/pkg	21247-20
Forceps, flat square tip		each	14537-00
Incubator, Dri-Bath, 25-well, 115/230 VAC		each	45900-00
Incubator, Dri-Bath, 25-well, 115/230 VAC, with European power cord		each	45900-02
Pipet, volumetric, Class A, 5.00-mL		each	14515-37
Pipet Filler, safety bulb		each	14651-00

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

3/04 1ed

TPH (Total Petroleum Hydrocarbons)

Method 10050 **Immunoassay Method***

Scope and Application: For soil and water

* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

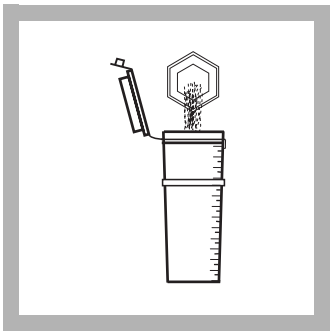
This TPH test can be used for both soil and water testing. When testing soil, purchase the necessary items (see *Required Apparatus on page 12*) and perform the *Soil Extraction Procedure*. When testing water samples only, proceed directly with *Immunoassay Procedure for Soil Extracts and Water Samples*.The test requires about 20 to 30 minutes for complete analysis. As many as 10 cuvettes can be run simultaneously.

Tips and Techniques

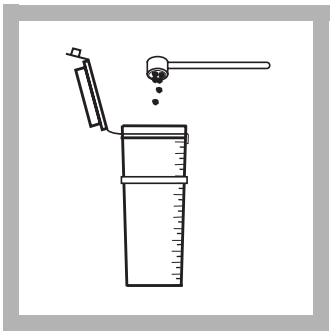
- Read the entire procedure before starting. Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis. A 1-cm square cell holder is required.
- Timing is critical; follow instructions carefully.
- A consistent technique when mixing the cuvettes is critical to this test. The best results come from using the cuvette rack and mixing as described in Using the 1-cm MicroCuvette Rack. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument.
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator or sample. Antibody Cuvettes are not reusable.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- Store the reagents at 4 °C (40 °F) when they are not in use. Allow the reagents to reach room temperature before using them in an analysis. Actual testing may be done at temperatures ranging from 1–38 °C (34–100 °F).
- The Soil Extractant contains methyl alcohol which is poisonous and flammable. Before using this and other reagents, read the Material Safety Data Sheet (MSDS) for proper use of protective equipment and other safety information.
- Hach Company recommends wearing protective nitrile gloves for this procedure.

Soil Extraction Procedure

1. Weigh out 10 g of soil in the plastic weighing boat.

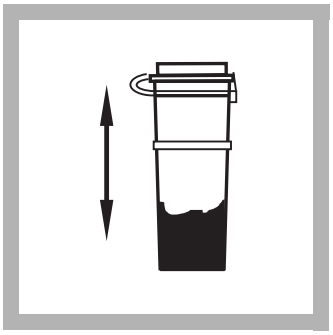


2. Carefully pour the soil into an extraction vial.



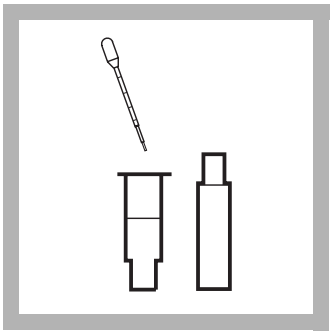
3. Use the 5-gram scoop to add one scoop of sodium sulfate to the extraction vial.

4. Use the graduated cylinder to transfer 10 mL of Soil Extractant into the extraction vial.



5. Cap the extraction vial tightly and shake vigorously for one minute.

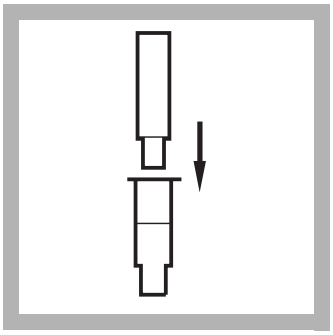
6. Allow to settle for at least one minute. Carefully open the extraction vial.



7. Using the disposable bulb pipet, withdraw 1.0–1.5 mL from the liquid layer at the top of the extraction vial.

Transfer it into the filtration barrel (the bottom part of the filtering assembly into which the plunger inserts).

Note: *Do not use more than 1.5 mL. The bulb is marked in 0.25-mL increments*

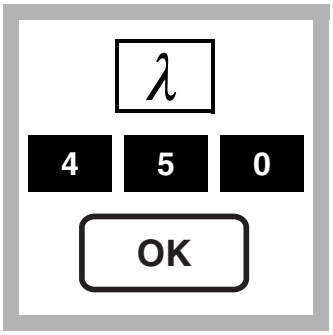


8. Insert the filtration plunger into the filtration barrel. Press firmly on the plunger until the sample extract is forced upward into the center of the plunger.

Use the resultant filtrate for the immunoassay in the*Immunoassay Procedure for Soil Extracts and Water Samples on page 3*.

Note: *It may be necessary to place the filtration assembly on a table and press down on the plunger.*

Immunoassay Procedure for Soil Extracts and Water Samples

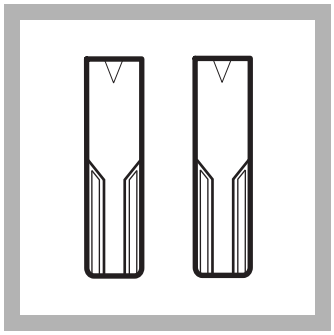


1. Touch

Single Wavelength

then touch the λ button. Type in 450 nm and touch **OK.**

Note: *Use the 1-cm square cell holder for this procedure.*

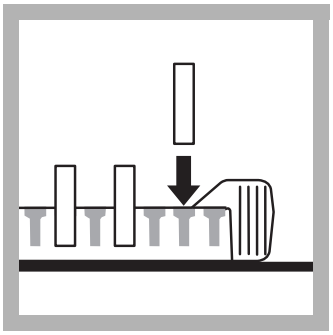


2. Label an Antibody Cuvette for each calibrator and each sample to be tested.

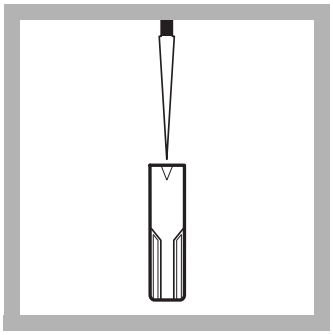
To select the proper calibrators, see *Table 1* on page *10* or *Table 2* on page *10*.

Note: *As many as 10 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.*

Note: *See example below.*



3. Place the cuvettes into the rack snugly.

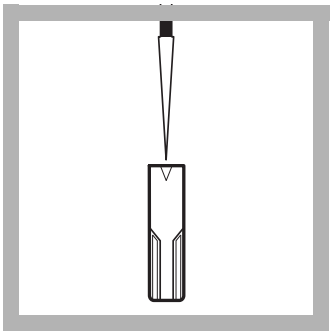


4. Pipet 0.5 mL of Diluent Solution into each Calibrator cuvette.

Note: *The same pipette tip can be used repeatedly for this step.*

EXAMPLE

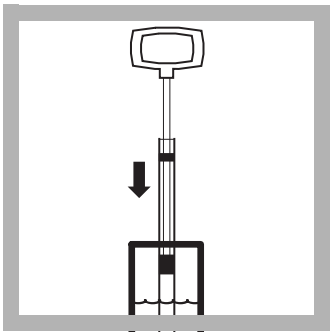
To test samples against the 50-ppm and 100-ppm diesel fuel calibrators, label one Antibody Cuvette “50” and a second cuvette “100.” Then label an Antibody Cuvette for each of up to eight samples to be tested. In this example, there is room for eight samples; samples plus calibrators cannot exceed 10. Using more calibrators will reduce the number of samples that can be run at the same time.



5. If testing soil: Pipet 0.5 mL of *Diluent Solution* into each sample cuvette.

If testing water: Pipet 0.5 mL of each *water sample* into the appropriate cuvette.

Note: *Use a new pipette tip for each water sample.*

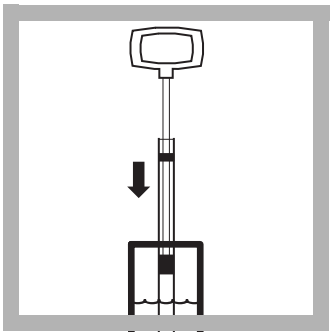


6. Have the necessary apparatus at hand for the next four steps as they must be done without delay.

Use a Wiretrol® pipet to transfer 50 µL of each calibrator to be used into the calibrator cuvettes.

Mix the contents of the cuvettes after each addition.

Note: *Use a separate capillary tube for each solution.*

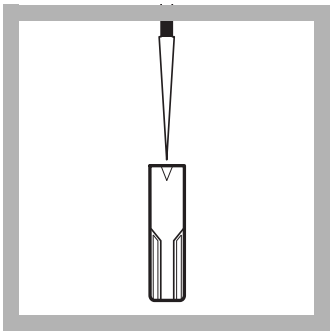


7. If testing soil: Use a Wiretrol pipet to transfer 50 µL of the filtered extract from *step 8* of the *Soil Extraction Procedure* into the appropriately labeled cuvette.

Note: *Use a separate capillary tube for each solution.*

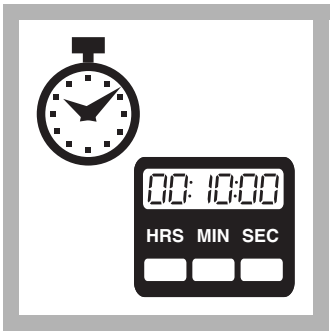
Mix the contents of the cuvettes after the addition of each sample.

If testing water: Use a Wiretrol pipet to transfer 50 µL of methanol into each sample cuvette



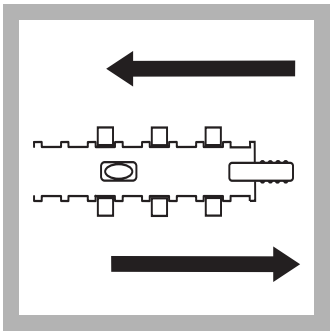
8. Immediately pipet 0.5 mL of TPH Enzyme Conjugate into each calibrator and sample cuvette.

Note: *The same pipette tip can be used repeatedly for this step.*



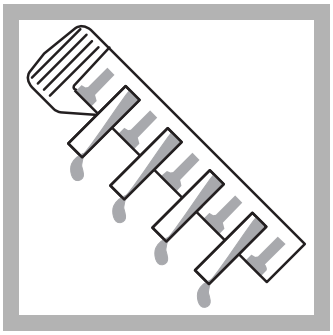
9. Touch the timer icon. Enter 10 minutes and press **OK.**

A 10-minute reaction time will begin. Proceed immediately to the next step.

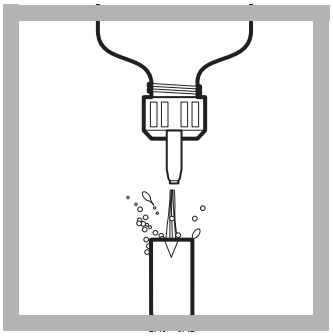


10. Mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

11. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.



12. At the end of the 10-minute period, discard the contents of all the cuvettes into an appropriate waste container.

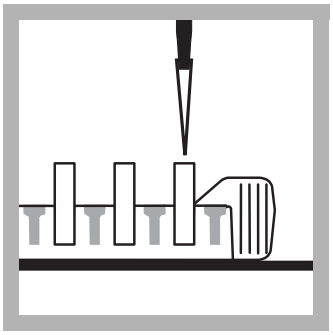


13. Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

Note: *Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel.*

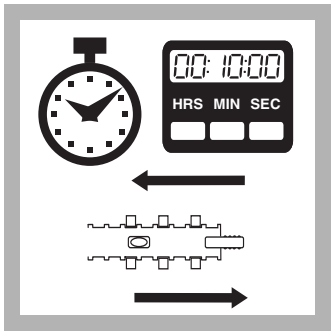
Color Development

Note: *Timing is critical; follow instructions carefully*



1. With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

Note: *Use a new pipette tip for each cuvette.*

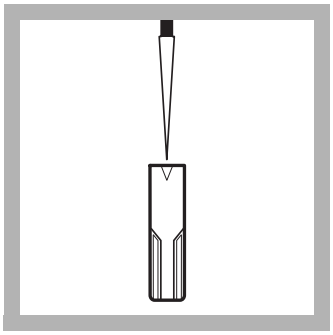


2. Touch the timer icon. Enter 10 minutes and touch **OK.**

A reaction period will begin. Mix following the instructions in *Using the 1-cm MicroCuvette Rack*.

3. After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

Note: *Solutions will turn blue in some or all of the cuvettes.*



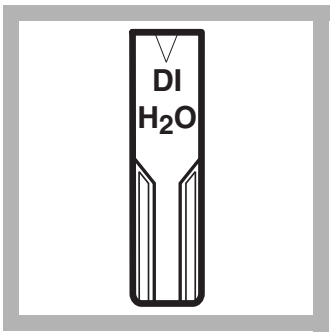
4. At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in *step 1*.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

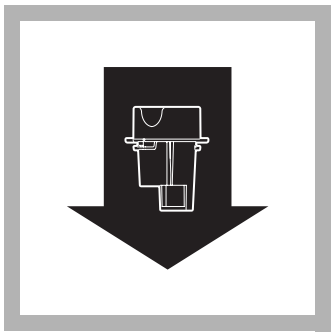
Note: *Blue solutions will turn yellow with the addition of the Stop Solution.*

Note: *The same pipette tip can be used repeatedly for this step.*

Measuring the Color



5. Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.

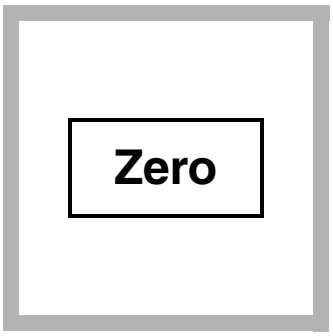


6. Install the 1-cm square cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

Place the filled zeroing cuvette into the cell holder—arrow pointing towards the left side of the instrument.

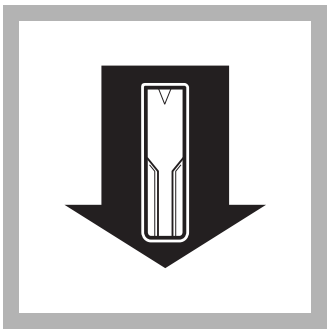
Orient the arrow in the same direction for all cuvettes.



7. Touch **Zero**.

The display will show:

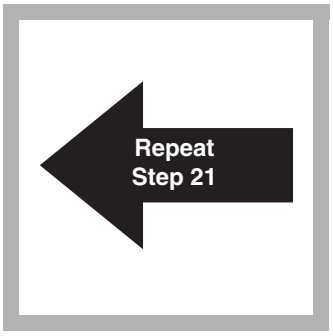
0.000 Abs



8. Place the first calibrator into the cell holder.

Touch **Read**.

The display will give an absorbance reading. Record the results for each calibrator and sample.



9. Repeat *step 8* for all remaining calibrators and samples.

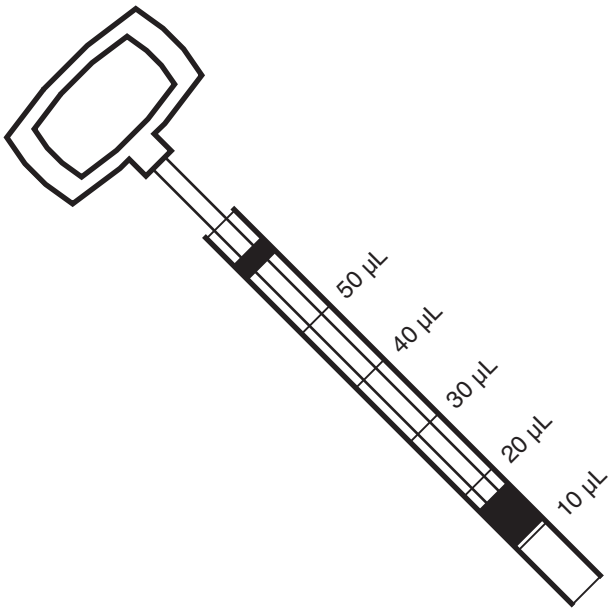
See *Interpreting and Reporting Results on page 8* for help with interpretation of results.

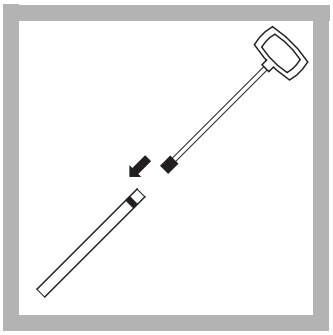
Using the Wiretrol® Pipet

The Wiretrol Pipet can accurately measure small quantities of liquids. It consists of two parts: a Teflon®-tipped plunger and a calibrated capillary tube. Use *Figure 1* to determine the quantity measured at each line on the capillary tube.

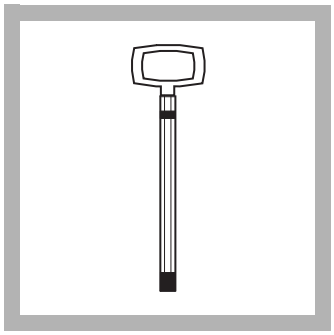
The plunger can be re-used; the capillary tubes must be discarded after one use.

Figure 1 Wiretrol Pipet

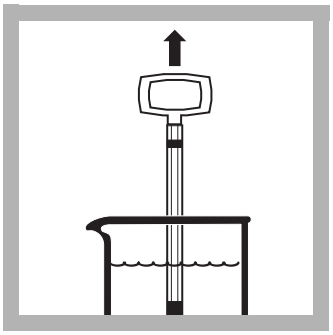




1. Wet the orange Teflon® tip of the Wiretrol plunger in the sample, and carefully insert it into the end of the capillary tube with the colored band on it.

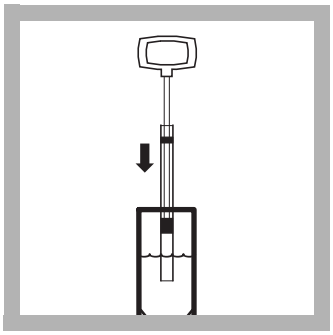


2. Push the tip to the other end of the capillary tube until it barely extends beyond the end of the capillary tube.



3. Submerge the capillary tube below the surface of the liquid to be pipetted. Slowly and smoothly draw the Wiretrol plunger up until the bottom of the plunger tip reaches the appropriate volume line.

Note: *Touch the end of the tube to the side of the vessel to release drops on the capillary tube tip.*

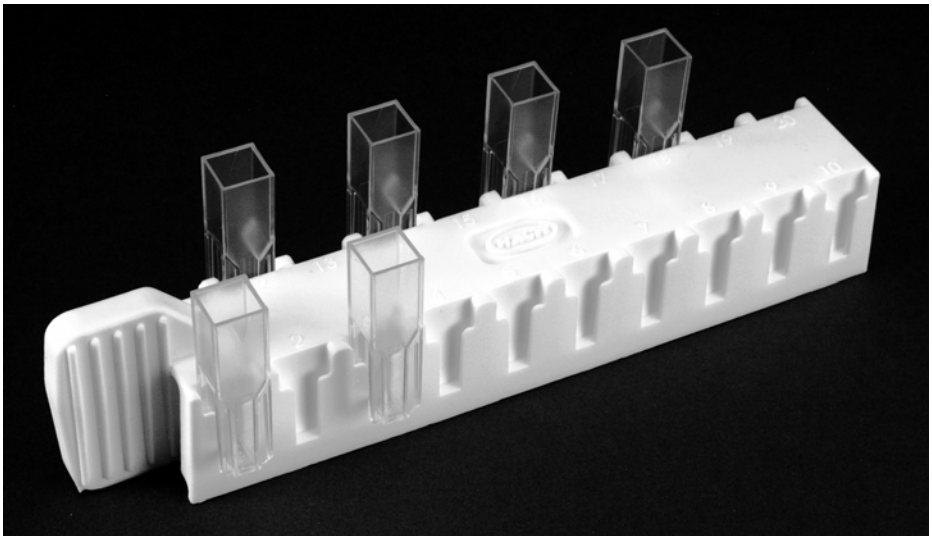


4. To discharge the pipet, place the tip of the capillary tube below the surface of the solution and push the Wiretrol plunger down in one smooth motion. Change capillary tubes for each calibrator and sample.

Using the 1-cm MicroCuvette Rack

This rack (see *Figure 2*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

Figure 2 The 1-cm MicroCuvette Rack



Loading the Rack — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

Mixing — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

Interpreting and Reporting Results

There is an inverse relationship between the concentration of TPH and the reading. In other words, the higher the reading, the lower the concentration of TPH.

If the sample reading is…	the sample TPH Concentration is…
…less than calibrator reading	…greater than the calibrator concentration
…greater than calibrator reading	…less than the calibrator concentration

Example

Readings:

TPH Calibrator #1: **0.480 Abs**

TPH Calibrator #2: **0.360 Abs**

Sample #1: **0.200 Abs**

Sample #2: **0.400 Abs**

Sample #3: **0.550 Abs**

Interpretation

Interpretation for a soil sample:

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of TPH is greater than both 20 ppm and 50 ppm diesel fuel.

Sample #2 — Sample reading is between the readings for the TPH calibrators. Therefore the sample concentration of TPH is between 20 ppm and 50 ppm diesel fuel.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of TPH is less than both 20 ppm and 50 ppm diesel fuel.

Interpretation for a water sample:

Sample #1 — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of TPH is greater than both 2 ppm and 5 ppm diesel fuel.

Sample #2 — Sample reading is between the readings for the TPH calibrators. Therefore the sample concentration of TPH is between 2 ppm and 5 ppm diesel fuel.

Sample #3 — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of TPH is less than both 2 ppm and 5 ppm diesel fuel.

Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store at a temperature of 4 °C (40° F) when not in use.
- Keep the foil pouch containing the TPH Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

Sensitivity

The antibodies used in the TPH Test Kit react with a variety of compounds found in petroleum fuels; however, each TPH calibrator has been formulated to represent a specific concentration of diesel fuel. To use the calibrators for other TPH compounds, see *Table 1* or *Table 2* to select the proper TPH calibrator for the compound, sample, and range you want to test.

Example:

To use the TPH calibrators for gasoline, find “Gasoline” in the first column of *Table 1* or *Table 2*. Read across the column to find the ppm represented by each calibrator. For gasoline, calibrator #1 = 15 ppm, calibrator #2 = 35 ppm, and so forth.

Table 1 Various TPHs in Soil

Compound	TPH calibrator #1	TPH calibrator #2	TPH calibrator #3	TPH calibrator #4
	ppm			
Diesel	20	50	100	200
Gasoline	15	35	70	140
Kerosene	35	75	140	250
Benzene	20	45	85	160
Toluene	15	30	50	90
Ethylbenzene	5	15	35	75
m-Xylene	9	20	35	70
o-Xylene	10	20	40	80
p-Xylene	3	5	9	16
BTEX	5	15	25	45

Table 2 Various TPHs in Water

Compound	TPH calibrator #1	TPH calibrator #2	TPH calibrator #3	TPH calibrator #4*
	ppm			
Diesel	2	5	10	20
Gasoline	1.5	3.5	7	14
Kerosene	3.5	7.5	14	25
Benzene	2	4.5	8.5	16
Toluene	1.5	3	5	9
Ethylbenzene	0.5	1.5	3.5	7.5
m-Xylene	0.9	2	3.5	7
o-Xylene	1	2	4	8
p-Xylene	0.3	0.5	0.9	16
BTEX	0.5	1.5	2.5	4.5

* To test concentrations in water higher than those covered by the calibrators, dilute the original sample as described below.

Diluting Water Samples

Higher concentrations in water can be tested by first diluting the sample with deionized water (see *Sensitivity*). Test for other TPH compounds (i.e., gasoline) by using the conversion factors given in *Table 1* and *Table 2*. Dilute the sample to 50 mL with deionized water in a graduated cylinder. (See *Reagent Set, TPH 20 cuvettes Cat. No. 27743-00*.)

Choose the mL of sample from *Table 3*. Use the multiplier value for the chosen quantity to multiply the value from *Table 2*, above.

Table 3

mL Sample	Multiplier
0.5	100
1.0	50
2.0	25
5.0	10
10.0	5
25.0	2

For example: If a 0.5 mL water sample is diluted to 50 mL and tested, the calibrator levels for diesel fuel in water would represent 200, 500, 1000, and 2000 ppm respectively.

Sample Collection and Storage

Analyze the samples as soon as possible after collection. If the samples must be stored, collect them in glass or Teflon® containers that have been washed with soap and water and rinsed with methanol. The container should be capped with a Teflon-lined cap. If a Teflon cap is not available, aluminum foil rinsed in methanol may be used as a substitute cap liner.

When collecting water samples, fill the container completely (no head space) and cover the container with a tightly-sealed lid immediately after collection.

For Soil: Store the samples at 4 °C (40 °F) for no longer than 14 days.

For Water: Chill the sample in an ice bath or refrigerator to limit the loss of volatile compounds. Store samples no longer than 24 hours.

Interferences

Interfering Substance	Interference Levels and Treatments
Chlorine in water samples	Interfere above 2 ppm Remove with sodium thiosulfate

Summary of Method

This method provides semi-quantitative screening based on thresholds for TPH as diesel fuel in the following concentrations:

Soil 20, 50, 100, 200 ppm as diesel fuel

Water 2, 5, 10, 20 ppm as diesel fuel

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Antibodies specific for TPH are attached to the walls of plastic cuvettes. They selectively bind and remove TPH from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and TPH compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by TPH and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of TPH in the sample. The resulting color is then compared with a calibrator to determine whether the TPH concentration in the sample is greater or less than the threshold levels. The TPH concentration is inversely proportional to the color development: the lighter the color, the higher the TPH concentration. Test results are measured at 450 nm.

Required Reagents

Description	Unit	Cat. No.
Deionized water	500 mL	272-48
Reagent Set, TPH*	20 cuvettes	27743-00

Required Apparatus

Cell holder, 1-cm square	each	59065-00
Caps, flip spout	2/pkg	25818-02
Marker, laboratory	each	20920-00
Rack, for 1-cm Micro Cuvettes	each	48799-00
TenSette, Pipet, 0.1–1.0mL	each	19700-01
Tips, for pipettor 19700-01	1000/pkg	21856-28
Wipes, disposable	box	20970-00
For Soil Extraction only:		
Soil Scoop, 5-g, 4.25-cc	each	26572-05
Soil Extraction Refill Kit	each	27752-00
Includes:		
Dropper, LDPE, 0.5 and 1.0-mL	20/pkg	21247-20
Filter and Barrel Assembly	20/pkg	25676-20
Sodium Sulfate, anhydrous	250 g	7099-14
Soil Extractant Solution	200 mL	25677-29
Soil Sample Container	20/pkg	25929-20
Spatula, disposable	2/pkg	25693-20
Weighing Boat, 8.9-cm square	20/pkg	21790-20

* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.

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FAX: (970) 669-2932

3/04 1ed

Volatile Acids

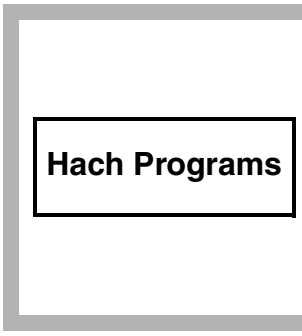
Method 8196

Esterification Method*
(27 to 2800 mg/L)

Scope and Application: For digestor sludges

* Adapted from *The Analyst*, 87, 949 (1962)

Method 8196



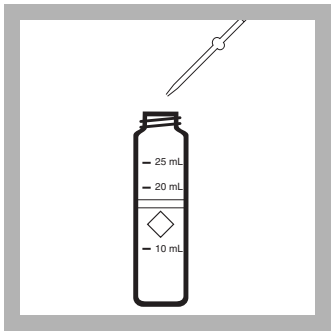
1. Touch

Hach Programs.

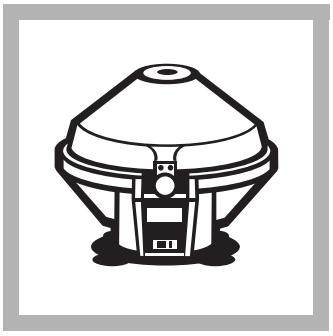
Select program

770 Volatile Acids.

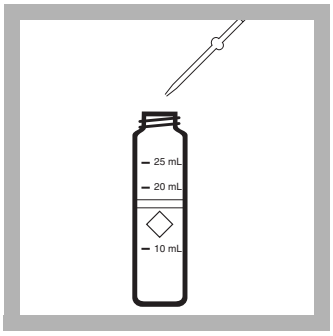
Touch **Start**.



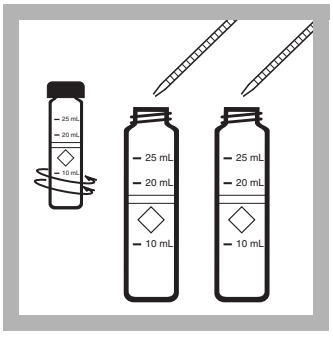
2. Pipet 0.5 mL of deionized water into a dry 25-mL sample cell (the blank).



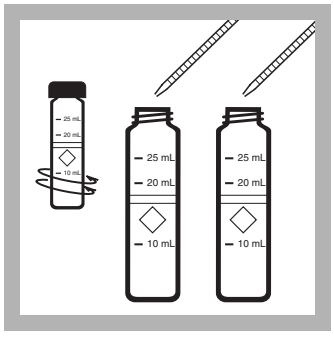
3. Filter or centrifuge 25 mL of sample. Centrifuging is faster than filtration.



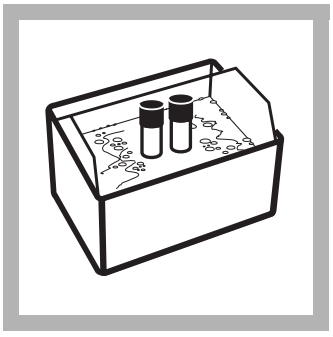
4. Pipet 0.5 mL of the filtrate or supernatant into a second dry 25-mL sample cell (the prepared sample).



5. Pipet 1.5 mL of ethylene glycol into each sample cell. Swirl to mix.

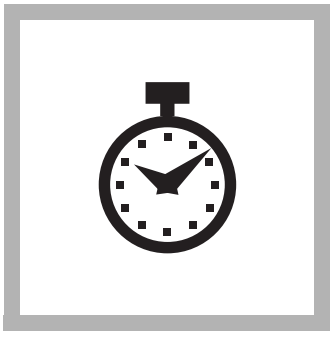


6. Pipet 0.2 mL of 19.2 N Sulfuric Acid Standard Solution into each cell. Swirl to mix.



7. Place both cells into a boiling water bath.

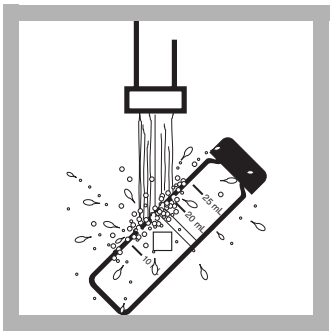
Alternatively, the cells may be boiled in a 500-mL beaker.



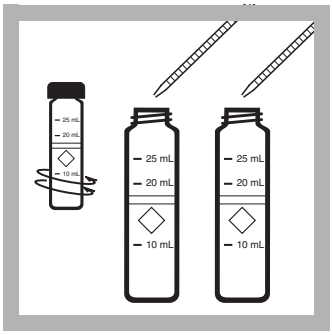
8. Touch the timer icon.

Touch **OK**.

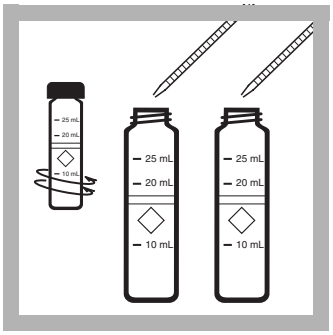
A three-minute reaction period will begin.



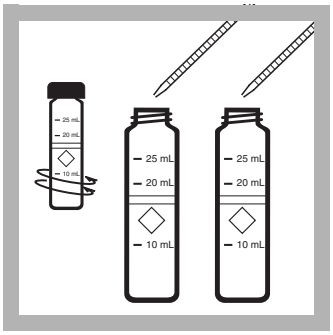
9. When the timer beeps, cool the solutions to 25 °C (until the cell feels cold) with running tap water.



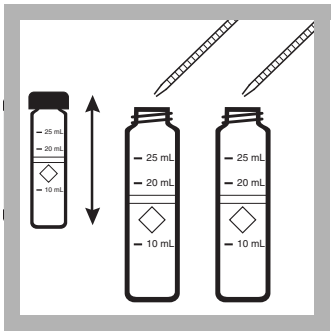
10. Using a pipet filler, pipet 0.5 mL of Hydroxylamine Hydrochloride Solution into each cell. Swirl to mix.



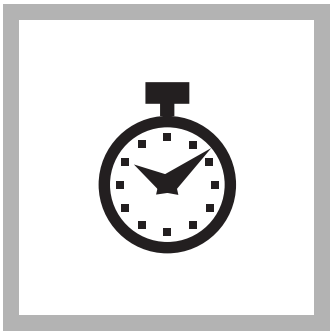
11. Using a pipet filler, pipet 2.0 mL of 4.5 N Sodium Hydroxide Standard Solution into each cell. Swirl to mix.



12. Add 10 mL of Ferric Chloride Sulfuric Acid Solution to each cell. Swirl to mix.



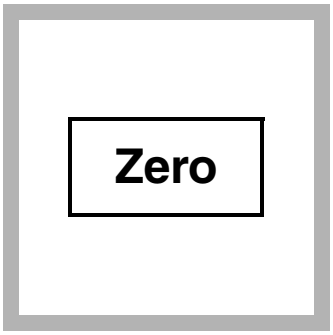
13. Add 10 mL of deionized water to each cell. Cap and invert to mix.



14. Immediately touch the timer icon. Touch **OK**.

Another three-minute reaction period will begin. During this time, complete steps *15* and *16*.

15. Blot each sample cell dry. Immediately place the blank into the cell holder.

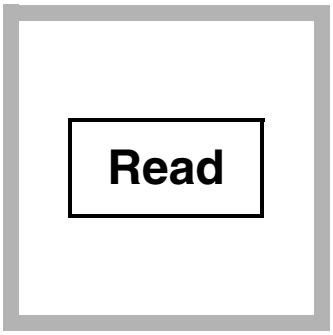


16. Touch **Zero**.

The display will show:

0 mg/L HOAC

17. Wipe the prepared sample and place it into the cell holder.



18. Touch **Read**.

Results will appear in mg/L HOAC.

Sample Collection, Preservation, and Storage

Collect samples in clean plastic or glass bottles. Analyze as soon as possible after collection. Samples can be stored for up to 24 hours by cooling to 4 °C (40 °F) or below. Warm samples to room temperature before analysis.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Volatile Acid Voluette® Ampule Standard, 62,500-mg/L as acetic acid.
5. Prepare three sample spikes. Fill three Mixing Cylinders (Cat. No. 1896-40) with 25 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 500 mg/L volatile acid standard solution as follows:

1. Pipet 4.00 mL of a 62,500-mg/L Volatile Acid Standard Solution into a 500-mL Class A volumetric flask. Dilute to volume with deionized water. Prepare this solution daily. Perform the esterification procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 500-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **OK** to accept the displayed concentration. If an alternate concentration is used, touch the number in the **Adjust to** field and enter the actual concentration. Touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision

Standard 625 mg/L as acetic acid (HOAC)

Program	95% Confidence Limits of Distribution
770	602–648 mg/L HOAC

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
625 mg/L	0.010	27 mg/L as HOAC

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

The volatile acid test is designed specifically for determining volatile acids in digestor sludges. The method is based on esterification of the carboxylic acids present in the sample and subsequent determination of the esters by the ferric hydroxamate reaction. All volatile acids present are reported as their equivalent mg/L as acetic acid. Test results are measured at 495 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Volatile Acids Reagent Set (90 tests)			22447-00
Includes:			
(1) Ethylene Glycol	3 mL	1000 mL	2039-53
(2) Ferric Chloride-Sulfuric Acid Solution	20 mL	1000 mL	2042-53
(1) Hydroxylamine Hydrochloride Solution, 100-g/L	1 mL	100 mL	818-42
(1) Sodium Hydroxide Standard Solution, 4.5 N	4 mL	1000 mL	2040-53
(1) Sulfuric Acid Standard Solution, 19.2 N	0.4 mL	100 mL MDB	2038-32
Water, deionized	20.5 mL	4 L	272-56

Required Apparatus

Centrifuge, 115 VAC, 60 Hz	1	each	26765-00
Centrifuge Tubes, 15-mL	2	10/pkg	22787-39
Centrifuge Tube Caps	2	20/pkg	25852-20
Cylinder, graduated, 10-mL	1	each	508-38
Filter Paper, folded, 12.5-cm		100/pkg	1894-57
Funnel, poly, 65-mm		each	1083-67
Hot Plate, 4-inch micro, 120 VAC	1	each	12067-01
Hot Plate, 4-inch micro, 240 VAC	1	each	12067-02
Pipet, Filler, safety bulb	1	each	14651-00
Pipet, serological, 2-mL	1	each	532-36
Pipet, volumetric, Class A, 0.50-mL	1	each	14515-34
Pipet, volumetric, Class A, 10.00-mL	1	each	14515-38
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06
Water Bath and Rack	1	each	1955-55

Required Standards

Volatile Acids Standard Solution,			
10-mL Voluette® Ampule, 62,500-mg/L as HOAC		16/pkg	14270-10

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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

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★Method 8009 — Zincon Method*

Powder Pillows — (0.01 to 2.00 mg/L)

Scope and Application: For water and wastewater; digestion is required for determining total zinc (see *Digestion on page 4*); USEPA Approved for wastewater analyses**

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** *Federal Register,* 45(105) 36166 (May 29, 1980).

Tips and Techniques

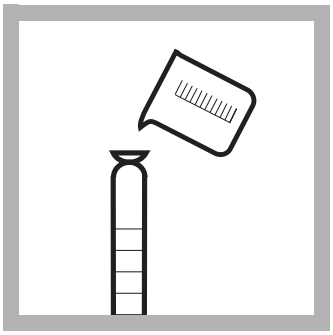
- Caution! ZincoVer® 5 Reagent contains cyanide and is very poisonous if taken internally or if fumes are inhaled. Do not add to an acidic sample (pH < 4).
- Use only glass-stoppered cylinders in this procedure.
- Wash glassware with 1:1 HCl (Cat. No. 884-49) and rinse with deionized water before use.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- Use plastic droppers in this procedure. Droppers with rubber bulbs may contaminate the reagent.
- ZincoVer 5 reagent contains potassium cyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Cyanide should be collected for disposal as a reactive (D003) waste. Be sure that cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See *Section 4* for further information on proper disposal of these materials.

Powder Pillows — Method 8009

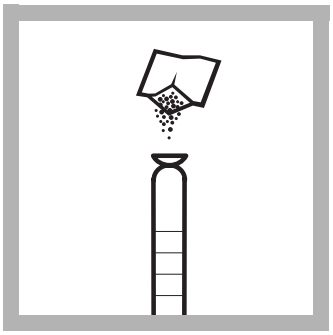
1. Touch **Hach Programs**.

Select program **780 Zinc**.

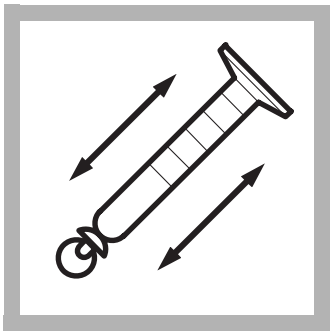
Touch **Start**.



2. Fill a 25-mL graduated mixing cylinder with 20 mL of sample.



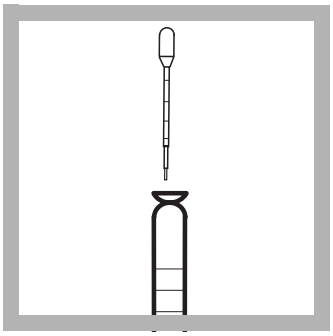
3. Add the contents of one ZincoVer 5 Reagent Powder Pillow to the cylinder. Stopper.



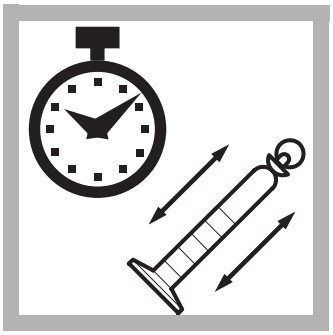
4. Invert several times to dissolve the powder completely. Inconsistent readings may result for low zinc concentrations if all the particles are not dissolved.

Note: *The sample should be orange. If the sample is brown or blue, either the zinc concentration is too high, or an interfering metal is present. Dilute the sample and repeat the test.*

5. Pour 10 mL of the solution into a sample cell (this is the blank).



6. Use a plastic dropper to add 0.5 mL of cyclohexanone to the remaining solution in the graduated cylinder.

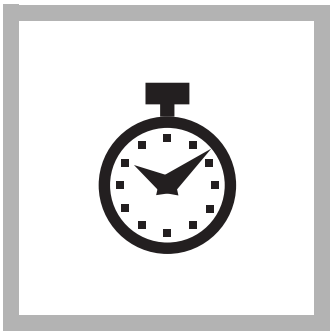


7. Touch the timer icon

Touch **OK**.

A 30-second reaction period will begin. During the reaction period, stopper the cylinder and shake vigorously (the prepared sample).

Note: *The sample will be reddish-orange, brown, or blue, depending on the zinc concentration.*



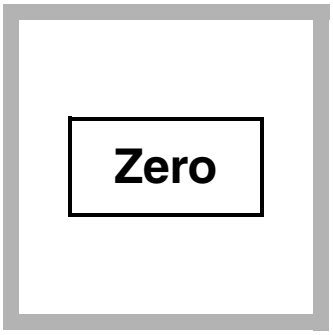
8. Touch the timer icon.

Touch **OK**.

A three-minute reaction period will begin. During this reaction period, complete *step 9*.

9. Pour the solution from the cylinder into a round sample cell (this is the prepared sample).

10. When the timer beeps, wipe the blank and place it into the cell holder.



11. Touch **Zero**.

The display will show:

0.00 mg/L Zn

12. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in mg/L Zn.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	Greater than 6 mg/L
Cadmium	Greater than 0.5 mg/L
Copper	Greater than 5 mg/L
Iron (ferric)	Greater than 7 mg/L
Manganese	Greater than 5 mg/L
Nickel	Greater than 5 mg/L
Organic Material	Large amounts may interfere. Pretreat the sample with a mild digestion.
Highly buffered or extreme sample pH	May exceed the buffering capacity of the reagents and require sample pretreatment. Adjust pH to 4–5.

Samples containing amino-tri(methylene phosphonic acid) (AMP) will exhibit a negative interference. Perform a total phosphorus digestion (Method 8190) to eliminate this interference. **IMPORTANT:** Adjust the pH of the sample after the total phosphorus digestion to 4–5 with Sodium Hydroxide before analysis with the zinc test.

Sample Collection, Preservation, and Storage

Collect samples in acid-cleaned plastic or glass bottles. If prompt analysis is impossible, preserve the sample by adjusting to pH 2 or less with nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.

Before analysis, adjust the pH to 4–5 with 5.0 N Sodium Hydroxide. Do not exceed pH 5 as zinc may precipitate. Correct the test result for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page *29*.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Snap the neck off a Zinc Voluette® Ampule Standard, 25-mg/L Zn.
5. Prepare three sample spikes. Fill three mixing cylinders (Cat. No. 20886-40) with 20 mL of sample and use the TenSette® Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively to each sample and mix thoroughly.
6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by touching **Read**. Each addition should reflect approximately 100% recovery.

7. After completing the sequence, touch **Graph** to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch **View: Fit**, then select **Ideal Line** and touch **OK** to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

Prepare a 1.00-mg/L zinc standard solution as follows:

1. Using Class A glassware, pipet 10.00 mL of Zinc Standard Solution, 100-mg/L, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the Zincon procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 1.00-mg/L Zinc standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Digestion

Digestion is required if total zinc is being determined. The following is not the USEPA digestion (see *Section 2.3 Digestion* for more information).

- **a.** If nitric acid has not been added to the sample previously, add 5 mL of Concentrated Nitric Acid (Cat. No. 152-49) to one liter of sample (use a glass serological pipet and pipet filler). If the sample was acidified at collection, add 3 mL of nitric acid to one liter of sample.
- **b.** Transfer 100 mL of acidified sample to a 250-mL Erlenmeyer flask.
- **c.** Add 5 mL of 1:1 Hydrochloric Acid (Cat. No. 884-49).
- **d.** Heat sample on a Hot Plate (Cat. No. 12067-01, -02) for 15 minutes at 95 °C (203 °F). Make sure the sample does not boil.
- **e.** Filter cooled sample through a membrane filter and adjust the volume to 100 mL with Deionized Water (Cat. No. 272-56).
- **f.** Adjust the pH to 4–5 with 5.0 N Sodium Hydroxide (Cat. No. 2450-26) before analysis (see *Sample Collection, Preservation and Storage* for instructions).

Method Performance

Precision

Standard 1.00 mg/L Zn

Program	95% Confidence Limits of Distribution
780	0.95–1.05 mg/L Zn

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.01 mg/L Zn

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Zinc and other metals in the sample are complexed with cyanide. Adding cyclohexanone causes a selective release of zinc. The zinc then reacts with 2-carboxy-2'-hydroxy-5'-sulfoformazyl benzene (zincon) indicator to form a blue-colored species. The blue color is masked by the brown color from the excess indicator. The intensity of the blue color is proportional to the amount of zinc present. Test results are measured at 620 nm.

Required Reagents

Description	Quantity Required Per Test	Unit	Cat. No.
Zinc Reagent Set, 20-mL sample size (100 tests = 100 samples + 100 blanks)			24293-00
Includes:			
Cyclohexanone	1 mL	100 mL MDB	14033-32
ZincoVer® 5 Reagent Powder Pillows	1 pillow	100/pkg	21066-69

Required Apparatus

Cylinder, graduated, mixing, 25-mL	1	each	20886-40
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

Required Standards

Water, deionized		4 L	272-56
Zinc Standard Solution, 100-mg/L		100 mL	2378-42
Zinc Standard Solution, 2-mL PourRite® Ampule, 25-mg/L as Zn		20/pkg	14246-20
Zinc Standard Solution, 10-mL Voluette® Ampule, 25-mg/L as Zn		16/pkg	14246-10

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PAR

UniCell™ Vials (0.10 to 6.00 mg/L Zn*)

Scope and Application: For waste water, drinking water, surface water, raw water, and process control

* Reagent sets for this method are only available in Europe.

Tips and Techniques

- Samples collected in glass or plastic bottles should be analyzed as quickly as possible.
- Adjust pH of preserved samples to between pH 3–5 with 1.0 N Sulfuric Acid Standard Solution before analysis.
- Clean the outside of the vial before placing it in the instrument. Wipe with a damp towel, followed by a dry one, to remove fingerprints and other marks.
- Use the standard solution or standard addition method to verify results. See *Accuracy Check*.
- See *Section 3.2.4* in the procedure manual for information on adjusting the calibration curve.
- The shelf life of test reagents can be extended to 24 months if kept at 4 °C (40 ° F).
- Make sure that the temperature of the water sample and the sample vial is between 15–25 °C (59–77 °F)
- Undissolved zinc and complexed zinc can only be determined after digestion using the Metal Prep Set (HCT 200). Total zinc measuring range is 0.12 –7.20 mg/L.
- **Underrange** appears on the display when the instrument is zeroed or when the determined sample concentration is below the operating range listed for this method.

UniCell Vials

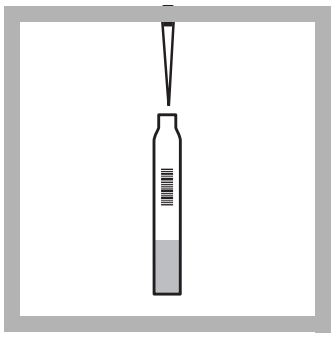
1. Touch

Hach Programs.

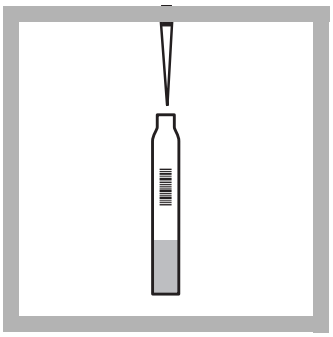
Select program

809 Zinc, HCT 170

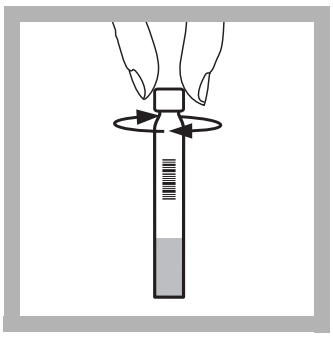
Touch **Start**.



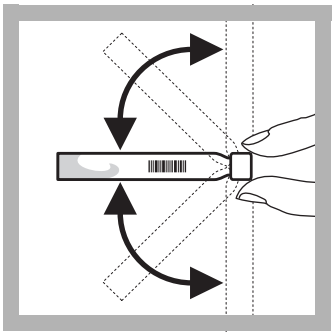
2. Pipet 4 mL of sample into a sample vial.



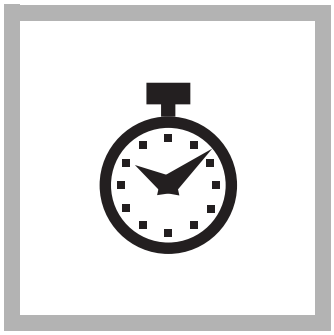
3. Pipet 0.4 mL of Demasking Solution A (HCT 170 A) into the sample vial.



4. **Immediately** cap with an **orange** UniCap 170 B.



5. Invert the sample vial repeatedly until the solids in the cap dissolve completely.

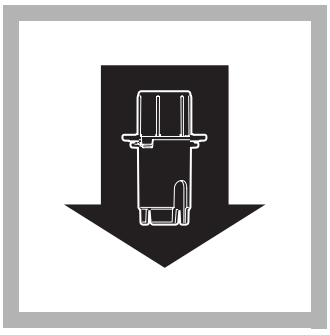


6. Touch the timer icon.

Touch **OK**.

An 3-minute reaction period will begin.

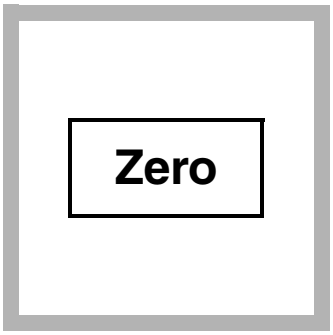
After the reaction period, invert the sample vial again to mix completely.



7. Install the 16-mm cell adapter.

Note: *See Section 2.6 Sample Cell Adapters in the Instrument Manual for adapter installation details.*

When the timer beeps, wipe the zero vial (**white** cap) and place it into the cell adapter.

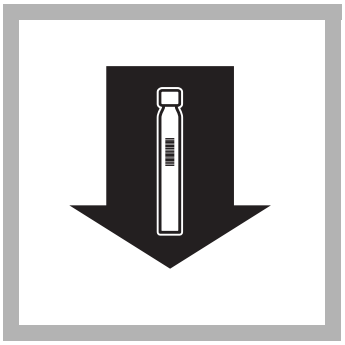


8. Touch **Zero**.

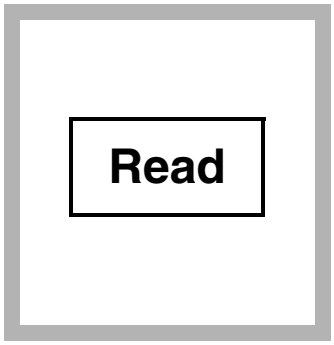
The display will show:

0.00 mg/L Free Zn
Underrange

Note: *If the sample was pretreated with the Metal Prep Set (HCT 200), Touch* ***Options*** *and select* ***Tot Zn****.*



9. Wipe the sample vial and place it into the cell adapter.



10. Touch **Read**.

Results will appear in mg/L Free Zn.

Interferences

The ions listed in the following table have been individually tested up to the given concentrations. Cumulative effects and the influence of other ions have not been evaluated.

Ion	No interference up to:
Cl^-, Ca^{2+}	1000 mg/L
Mg^{2+}	500 mg/L
Fe^{2+}, Fe^{3+}, Sn^{2+}, Ni^{2+}, Cu^{2+}, Cr^{3+}, Cr^{6+}, CO_3^{2-}	50 mg/L
Co^{2+}	20 mg/L
Pb^{2+}	5 mg/L

Sample Collection, Preservation, and Storage

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the samples immediately. To preserve samples, adjust the pH to 2 or less with concentrated nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature. If reporting only dissolved free zinc, filter sample immediately after collection and before adding nitric acid.

Accuracy Check

Standard Additions Method (Sample Spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Touch **Options**. Touch **Standard Additions**. A summary of the standard additions procedure will appear.
3. Touch **OK** to accept the default values for standard concentration, sample volume, and spike volumes. Touch **Edit** to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See *Standard Additions* in the instrument manual for more information.
4. Prepare three sample spikes by filling three mixing cylinders with 100 mL of sample. Pipet 0.1, mL, 0.2 mL, and 0.3 mL of 1000-mg/L Zn standard, respectively, to each sample and mix thoroughly.
5. Transfer 4 mL of each solution into a sample vial and analyze as described in the procedure. Touch **Read** to accept each standard additions reading. Each addition should reflect approximately 100% recovery.

See *Section 3.2.2 Standard Additions* on page *32* for more information.

Standard Solution Method

1. Prepare a 2.00-mg/L Zn Standard Solution by pipetting 0.2 mL of Zinc Standard Solution, 1000-mg/L, into a 100-mL volumetric flask. Dilute to the mark with deionized water. Stopper and invert to mix. Prepare this solution daily. Perform the zinc procedure as described above.
2. To adjust the calibration curve using the reading obtained with the 2.00-mg/L standard solution, touch **Options** on the current program menu. Touch **Standard Adjust**.
3. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected chemical form). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.4 Adjusting the Standard Curve* on page *40* for more information.

Method Performance

Precision
Standard: 2.00 mg/L Free Zn

Program	95% Confidence Limits of Distribution
809	1.63–2.37 mg/L Free Zn

See *Section 3.4.3 Precision* on page *44* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

Program	Portion of Curve	ΔAbs	ΔConcentration
809	1.00	0.010	0.085 mg/L Free Zn
809	3.00	0.010	0.087 mg/L Free Zn
809	5.00	0.010	0.090 mg/L Free Zn

See *Section 3.4.5 Sensitivity* on page *44* for more information.

Summary of Method

Zinc ions form a water soluble orange-red complex with 4-(2-pyridylazo)-resorcinol (PAR) at pH 5. The results are measured at 490 nm.

Required Reagents

Description	Unit	Cat. No.
Zinc - Zn, UniCell™ HCT 170*	23/pkg	HCT 170

Optional Apparatus

Flask, volumetric, 100-mL	each	14574-42
Graduated cylinder, mixing, 100-mL	each	20886-42
Pipettor, (Jencons) 1–5 mL	each	27951-00
Replacement tips for 27951-00	pk/100	27952-00
Pipettor, (Jencons) 100–1000 µL	each	27949-00
Replacement tips for 27949-00	pk/400	27950-00
pH Paper	pk/100	26013-00

Required Apparatus

Adapter, 16-mm Cell	each	59457-00

Optional Reagents

Nitric Acid Solution, 1:1	500 mL	2540-49
Sulfuric Acid Standard Solution, 1.00 N	1 L	1270-53
Sodium Hydroxide, 5 N	1 L	2450-53
Zinc Standard Solution, 1000-mg/L as Zn	100 mL	14177-42

* Available in Europe only

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