



Method 10127

Molybdovanadate Method

with Acid Persulfate Digestion*

Test 'N Tube™ Vials

HR (0.0 to 100.0 mg/L PO₄³⁻)

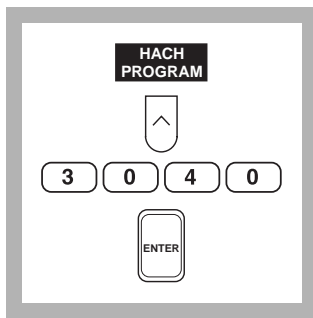
Scope and Application: For water and wastewater

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



1. Turn on the COD Reactor. Heat to 150 °C. Place the plastic shield in front of the reactor.

Note: Ensure safety devices are in place to protect the analyst if splattering or leakage occurs.



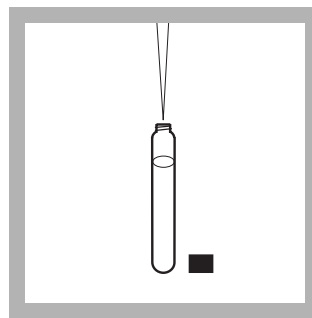
2. Press the soft key under **HACH PROGRAM**. Select the stored program number for phosphorus, total, high range, Test 'N Tube. Press: **3040 ENTER**

Note: If samples cannot be analyzed immediately, see *Sampling and Storage* following these steps.

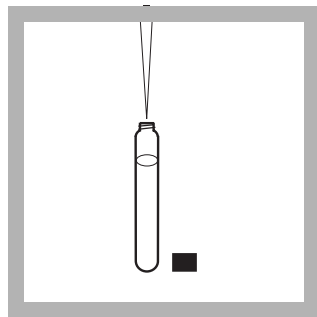


3. The display will show: **HACH PROGRAM: 3040 P Total HR TNT**

The wavelength (λ), **420 nm**, is automatically selected.

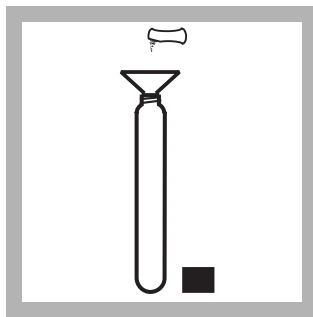


4. Use a TenSette® Pipet to add 5.0 mL of deionized water to a Total Phosphorus Test 'N Tube Vial (the blank).



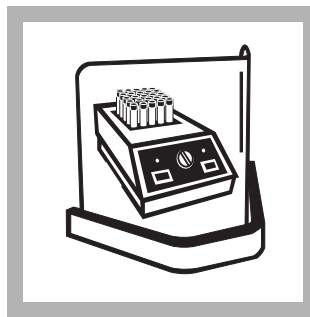
5. Use a TenSette Pipet to add 5.0 mL of sample to a Total Phosphorus Test 'N Tube Vial (the sample).

Note: For non-preserved samples with extreme pH, see *Interferences* following these steps.

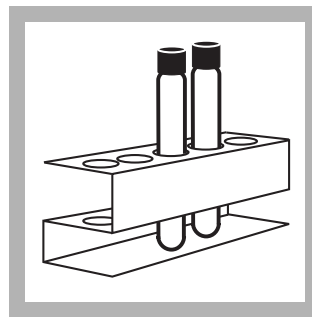


6. Use a funnel to add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to each vial. Cap tightly and shake to dissolve.

Cap tightly and shake to dissolve.



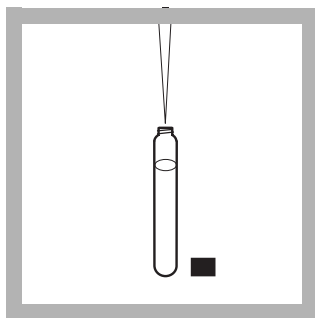
7. Place the vials in the COD Reactor, and start a 30-minute heating period by pressing the soft key under **START TIMER**.



8. Carefully remove the vials from the reactor. Place them in a test tube rack and allow to cool to room temperature (18-25 °C).

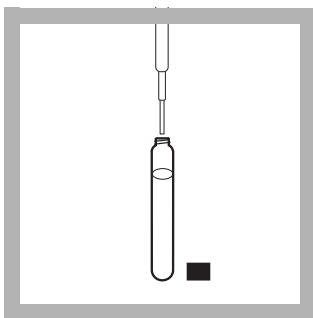
Note: Vials will be hot.

PHOSPHORUS, Total, continued



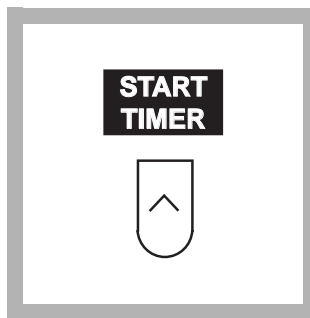
9. Use a TenSette Pipet to add 2.0 mL of 1.54 N sodium hydroxide to each vial.

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.

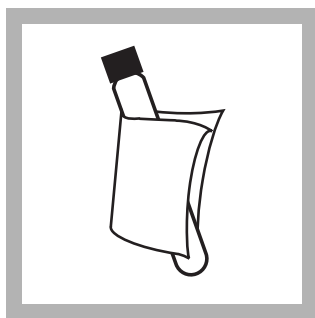


11. Press the soft key under **START TIMER**. A 7-minute reaction period will begin.

Note: Read the sample between 7 and 9 minutes.

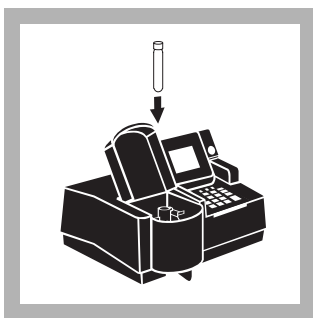


12. Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



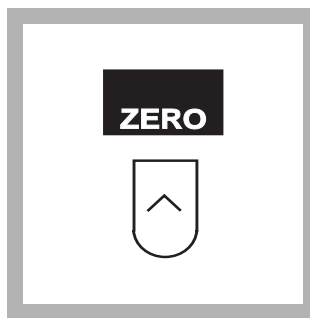
13. Clean the outside of the vials with a towel.

Note: Wipe with a damp towel, followed by a dry one, to remove fingerprints or other marks.



14. When the timer sounds, place the blank vial in the cell holder and close the light shield.

Note: Reagent blanks for each lot of reagents may be used more than once, but should not be used for longer than one day.

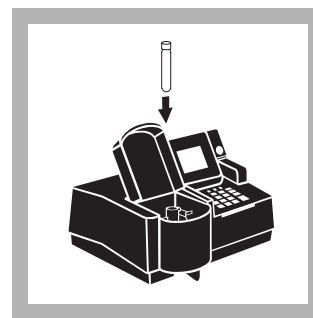


15. Press the soft key under **ZERO**.

The display will show:

0.0 mg/L PO₄³⁻

Note: For alternate concentration units, press the soft key under **OPTIONS**. Then press the soft key under **UNITS** to scroll through the available options. Press **ENTER** to return to the read screen.



16. Place the prepared sample vial into the cell holder and close the light shield. Results in mg/L PO₄³⁻ (or chosen units) will be displayed.

Note: Results may be expressed as phosphorus (P) or as phosphorus pentoxide (P₂O₅). Press the soft keys under **OPTIONS** and then **FORM**: to scroll through the available options.

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:

PHOSPHORUS, Total, continued

Interfering Substance	Interference Level and Treatment
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 <i>pH Interference</i> in Section 1.3 of the <i>DR/4000 Procedures Manual</i> . 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)	Causes 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 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 H₂SO₄ (about 2 mL per liter) and storing at 4 °C. Warm the sample to room temperature and neutralize with 5.0 N NaOH before analysis. Correct for volume additions; see *Section 1, Correcting for Volume Additions*.

Accuracy Check

Standard Additions Method

Note: Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use detergents containing phosphate to clean glassware.

- a. Leave the unspiked sample (from *step 16* in the procedure) in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under **OPTIONS, (MORE)** and then **STD ADD**.
- b. Press **ENTER** to accept the default sample volume (mL), 10.
- c. Press **ENTER** to accept the default standard concentration, 500 mg/L as PO₄³⁻.
- d. Press the soft key under **ENTRY DONE**.
- e. Fill each of three 10-mL graduated mixing cylinders with 10 mL of sample.
- f. Snap the neck off a 10-mL Voluette Ampule of Phosphate Standard Solution, 500 mg/L as PO₄³⁻ (Cat. No. 14242-10).

- g. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples from *step e*. Mix well.
- h. Analyze each standard addition sample from *step g* as described in the procedure. Accept the standard additions reading by pressing the soft key under **READ** each time. Each addition should reflect approximately 100% recovery, or an increase of 5 mg/L PO₄³⁻ for each 0.1 mL of standard added.
- i. 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.
- j. See *Section 1.4.1, Standard Additions*, for more information.

Standard Solution Method

To check accuracy, prepare a 80 mg/L standard by pipetting 8.0 mL of solution from a 10-mL Voluette Ampule of Phosphate Standard Solution, 500-mg/L as PO₄³⁻, into an acid-cleaned, 50-mL Class A volumetric flask. Dilute to the mark with deionized water. Substitute this standard for the sample and perform the procedure as described.

Standard Adjust

To adjust the calibration curve using the reading obtained with the 80 mg/L PO₄³⁻ Phosphate Standard Solution, press the soft keys under **OPTIONS, (MORE)** then **STD:OFF**. Press **ENTER** to accept the default concentration, 80.0 mg/L PO₄³⁻. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. For more information, see *Section 1.5.5, Adjusting the Calibration Curve*.

Method Performance

Precision

Standard: 80.0 mg/L PO₄³⁻

Program	95% Confidence Limits
3040	78.0–82.0 mg/L PO ₄ ³⁻

For more information on determining precision data and method detection limits, refer to *Section 1.5, Estimated Detection Limit*.

Estimated Detection Limit

Program	EDL
3040	5.0 mg/L PO ₄ ³⁻

For more information on derivation and use of Hach’s estimated detection limit, see *Section 1.5.2*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, appendix B, see *Section 1.5.1*.

Sensitivity

Program Number: 3040

Portion of Curve	Δ Abs	Δ Concentration
Entire Range	0.010	0.9 mg/L

See Section 1.5.3, *Sensitivity Explained*, for more information.

Calibration Standard Preparation

To perform a phosphate calibration using the Total High Range Phosphorus Test 'N Tube method, prepare calibration standards containing 10, 25, 50, 75, and 100 mg/L phosphate as follows:

- a. Into a 500-mL Class A volumetric flask, pipet 10.0 mL of 500 mg/L Phosphate Standard Solution using a Class A pipet.
- b. Into four separate 100-mL Class A volumetric flasks, pipet 5.0, 10.0, 15.0 and 20.0 mL of a 500-mg/L Phosphate Standard Solution (Cat. No. 14242-32) using Class A glassware.
- c. Dilute to the mark with deionized water. Mix thoroughly.
- d. Using the Total High Range Phosphorus Test 'N Tube method and the calibration procedure described in the *User-Entered Programs Section* of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the Material Safety Data Sheet for information specific to the reagents used.

Sample Disposal Information

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.

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 phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration.

PHOSPHORUS, Total, continued

REQUIRED REAGENTS

Total High Range Phosphorus Test 'N Tube Reagent Set.....50 vials.....27672-45
Includes: (50) Total Phosphorus Test 'N Tube Vials*, (2) 272-42, (1) 20847-66, (1) 20760-26, (1) 27430-42

Description	Quantity Required		Cat. No.
	Per Test	Unit	
Molybdovanadate Reagent.....	0.5 mL.....	25 mL.....	20760-26
Potassium Persulfate powder Pillows.....	1	50/pkg.....	20847-66
Sodium Hydroxide Solution, 1.54 N.....	2 mL.....	100 mL.....	27430-42
Total Phosphorus Test Vials	1	50/pkg.....	*
Water, deionized.....	100 mL.....	272-42

REQUIRED APPARATUS

COD Reactor, 115/230 VAC (U.S.A. and Canada).....	1	each.....	45600-00
COD Reactor, 115/230 VAC (Europe)	1	each.....	45600-02
DR/4000 Test Tube Adapter.....	1	each.....	48189-00
Pipet, TenSette, 1 to 10 mL.....	1	each.....	19700-10
Pipet Tips, for 19700-10 TenSette Pipet	1	1000/pkg.....	21997-28
Safety Shield, laboratory bench	1	each.....	23810-00
Test Tube Rack.....	1-3.....	each.....	18641-00

OPTIONAL REAGENTS

Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phosphate Standard Solution, PourRite Ampule, 500 mg/L as PO_4^{3-} , 2-mL	20/pkg.....	14242-20
Phosphate Standard Solution, Voluette ampule, 500 mg/L as PO_4^{3-} , 10 mL.....	16/pkg.....	14242-10
Phosphate Standard Solution, Voluette ampule, 500 mg/L as PO_4^{3-} ,	100 mL MDB.....	14242-32
Sodium Hydroxide Standard Solution, 5.0 N.....	1 liter.....	2450-53
Sulfuric Acid, ACS, concentrated	500 mL.....	979-49

OPTIONAL APPARATUS

Ampule Breaker Kit	each.....	21968-00
Aspirator, vacuum	each.....	2131-00
Cylinder, graduated, mixing, 10-mL, 3 required.....	each.....	20886-38
Filter Holder, 47-mm, 300-mL, graduated	each.....	13529-00
Filter Membrane, 47-mm, 0.45-microns	200/pkg.....	13530-01
Flask, filtering, 500-mL	each.....	546-49
Flask, volumetric, Class A, 50-mL	each.....	14574-41
Flask, volumetric, Class A, 100 mL.....	each.....	14574-42
Flask, volumetric, Class A, 500 mL.....	each.....	14574-49
pH Indicator Paper, 1 to 11 pH units.....	5 rolls/pkg.....	391-33
pH Meter, <i>sensio</i> TM I, portable	each.....	51700-10
Pipet Filler, Safety Bulb	each.....	14651-00
Pipet, TenSette, 0 to 1.0 mL.....	each.....	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	50/pkg.....	21856-96
Pipet, volumetric, Class A, 5.00 mL	each.....	14515-37
Pipet, volumetric, Class A, 8.00-mL.....	each.....	14515-08
Pipet, volumetric, Class A, 10.0-mL.....	each.....	14515-38
Pipet, volumetric, Class A, 15.0-mL.....	each.....	14515-39
Pipet, volumetric, Class A, 20.0-mL.....	each.....	14515-20

* These items are not sold separately.



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