TOXICITY

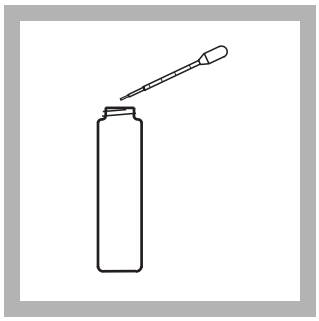
Method 10017

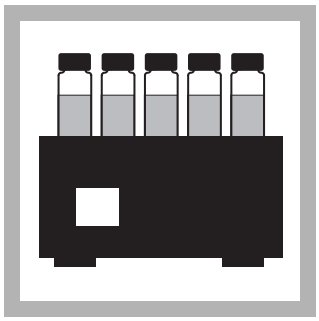
ToxTrak Method

(0 to 100% Inhibition)

Scope and Application: *For water and wastewater*

Inoculum Development Using Indigenous Biomass



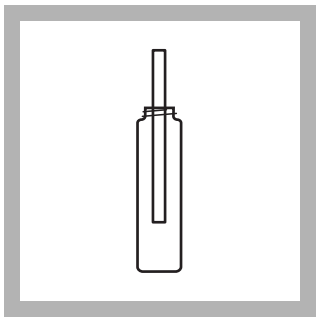


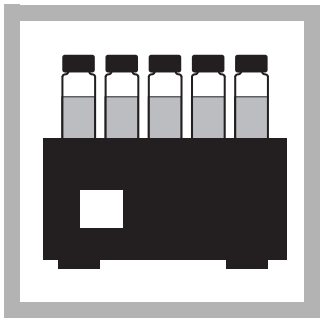
Using Indigenous Biomass

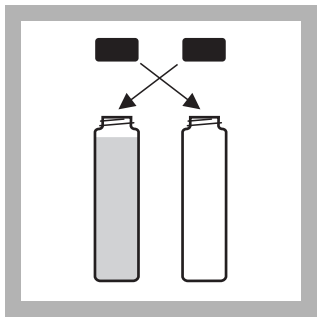
1. Using one of the dropper pipets provided, add 1.0 mL of source culture to a Tryptic Soy Broth Tube.

2. Incubate until the vial contents are visibly turbid (turbidity indicates bacterial growth).

Inoculom 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 until the medium is visibly turbid. Turbidity will develop much faster if incubation is done at 35 °C instead of room temperature. At 35 °C, 12 hours is usually sufficient.

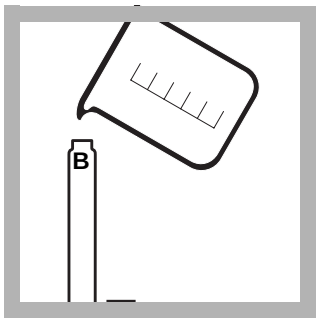
Note: *If toxicity tests will be run on consecutive days, inoculum may be kept several days at room temperature.*

3. Inoculate a new Lauryl Tryptose Broth Tube by first inverting the tube and switching the caps of the two tubes. Then invert the new tube. After incubation, this new vial may be used in subsequent tests.

Note: *In this way, several medium vials may be inoculated from a single initial tube.*

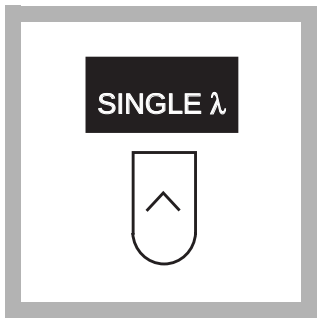
Note: *Cultures 10 to 72 hours old give best results.*

4. Insert the COD sample cell adapter into the sample cell compartment.



5. Fill a Test ‘N Tube sample cell with deionized water. This is the blank.

6. Place the blank into the cell adapter. Close the light shield.



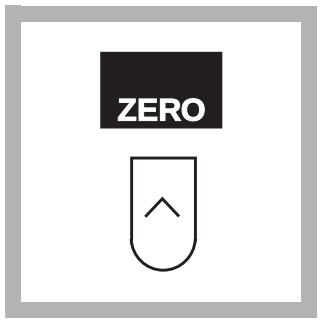
7. Press the soft key under ***SINGLE*** λ**.**

Press the soft key under ***GO TO*** λ.

Select 603 nm by pressing the numeric keys **6 0 3**.

Press: **ENTER**

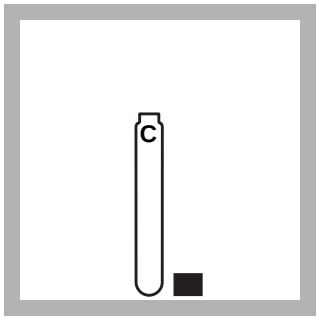
Note: *The Flow Cell and Sipper Modules cannot be used with this procedure.*



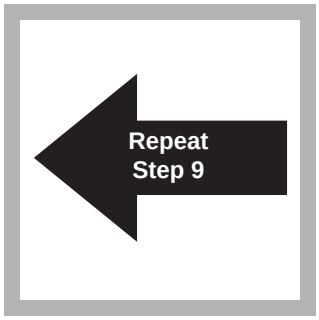
8. Press the soft key under ***ZERO***.

The display will show:

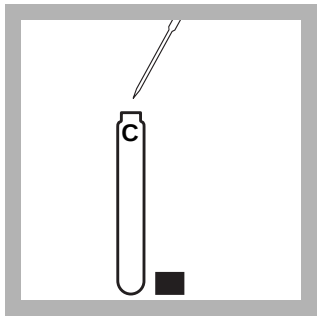
0.000 ABS



9. Label a cell “Control.” Open one ToxTrak Reagent Powder Pillow and add the contents to the empty reaction cell.

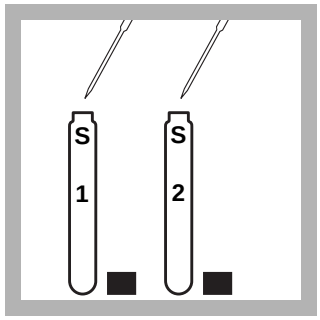


10. For each sample or dilution, repeat Step 9. Label each cell clearly.



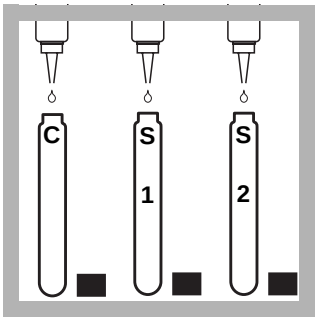
11. Add 5.0 mL of deionized water to the Control cell.

Note: *For a Control cell, use deionized water that is free of toxicity, or use another water source that represents baseline toxicity.*



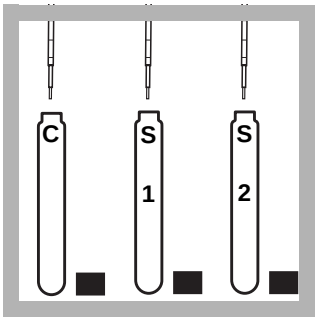
12. Add 5.0 mL of sample (or dilutions) to each sample cell.

Note: *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 which gives 0% Inhibition in final calculation.*



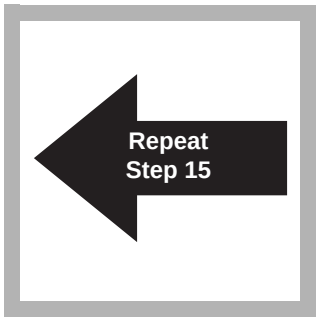
13. Add 2 drops of Accelerator Solution to each tube. Cap and shake to mix.

Note: *Shaking serves to fully oxygenate the samples, assuring that oxygen concentration is not a factor in determining respiration rate.*



14. Add 0.5 mL of inoculum (previously prepared) to each tube. Cap and invert to mix.

15. Place the Control into the adapter. Close the light shield. Record the absorbance.

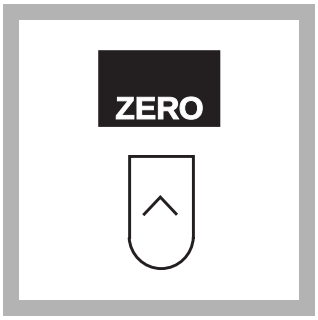


16. Repeat Step 15 for all samples and dilutions. Be sure to record each absorbance.

17. 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.

Note: *Reaction time varies according to temperature, age of the culture, bacteria concentrations, etc.*

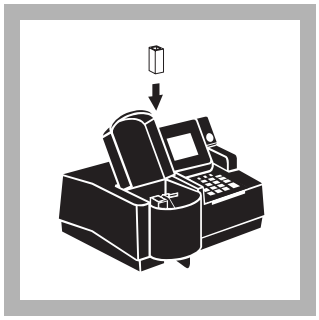
18. After the absorbance of the Control has decreased to 0.60 ±0.10 abs., place the Blank into the adapter and close the light shield.



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

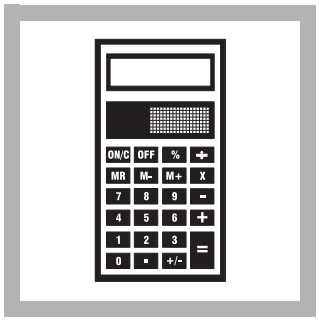
The display will show:

0.000 ABS



20. Place the Control into the adapter and close the light shield. Record the absorbance value of the Control.

21. Place each sample or dilution into the adapter and close the light shield. Record each absorbance value.



22. Calculate the % Inhibition as follows:

$$\%I = [1 - \Delta A_{sample} \div \Delta A_{control}] \times 100$$

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 in step 18 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 Observed 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:

1. Autoclave used test containers at 121 °C 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.

2. 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.

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. For additional information, refer to *Section 1*.

Pollution Prevention and Waste Management

For information on pollution prevention and waste management, refer to *Section 1*.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
ToxTrak Reagent Set (25 tests)	25972-00

Includes all consumable reagents and apparatus used in the test

Description	Quantity Required per test	Unit	Cat. No.
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
Water, deionized	varies	200 mL	272-29
Aqua QC-Stiks, *Escherichia coli*	1	3 cultures	27063-03

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, to open powder pillows	each	936-00
Dropper, 0.5- and 1.0-mL marks	20/pkg	21247-20
DR/4000 1-cm Adapter, square	each	48584-00
Forceps, flat square tip	each	14537-00
Pipet, volumetric, class A, 5.00 mL	each	14515-37
Pipet Filler, safety bulb	each	14651-00
Sample Cell, 9.5 x 1 x 1 cm, with cap	10/pkg	26275-10

OPTIONAL REAGENTS AND STANDARDS

Culture Set (Bactrol Disks and Lauryl Tryptose Broth Tubes)	25 cultures	25978-00

OPTIONAL EQUIPMENT AND SUPPLIES

Alcohol Burner, 60-mL	each	20877-60
Bunsen Burner, propane	each	21627-00
Germicidal Cloth	50/pkg	24632-00
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
Test Tube Rack, 13-mm, 6 x 15 rows	each	24979-00

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