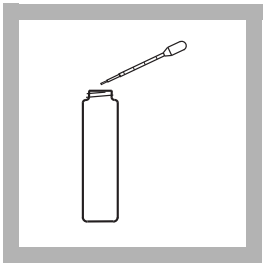
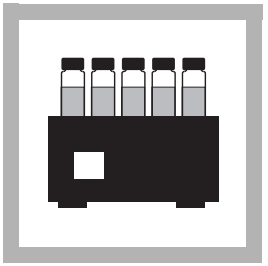
TOXTRAK™ TOXICITY TEST*

Colorimetric Method**

Inoculum Development



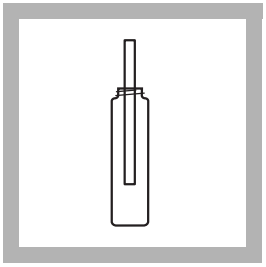


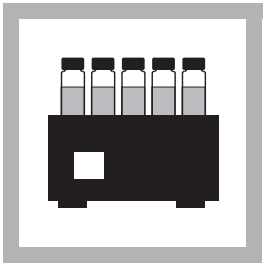
Using Indigenous Biomass

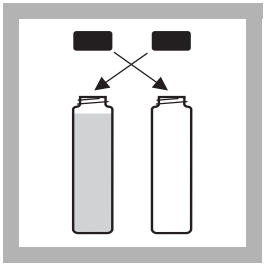
1. Using one of the dropper pipets provided, add 1.0 mL of source culture to a Tryptic Soy Broth Tube.

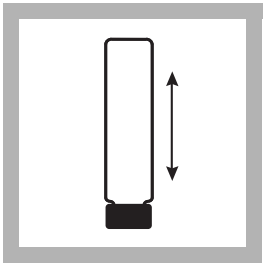
2. Incubate at 37 °C until the vial contents are visibly turbid (turbidity indicates bacterial growth).

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 (approxamately 12 hours). Turbidity develops much faster in an 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.

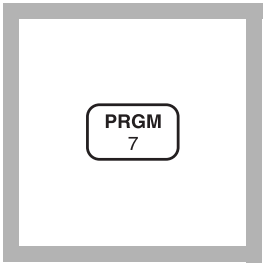
If toxicity tests will be run on consecutive days, inoculum may be kept several days at room temperature.

Cultures 10 to 72 hours old give best results.

* U.S. Patent number 5,413,916.

** Liu, D., *Bull. Environ. Contam. Toxicol.* 26, 145-149 (1981).

Colorimetric Reaction

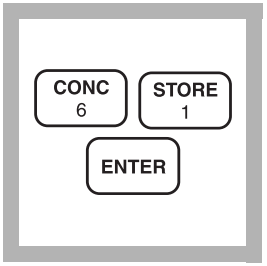


1. Enter the stored program number for toxicity.

Press: **PRGM**

The display will show:

PRGM ?

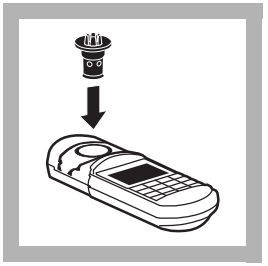


2. Press: **61 ENTER**

The display will show:

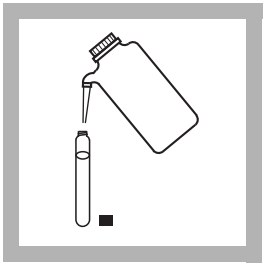
ABS 610 nm

and the zero icon.

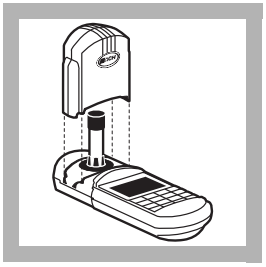


3. Insert the TNT/COD Adapter into the cell holder by rotating the adapter until it drops into place. Then push down to fully insert it.

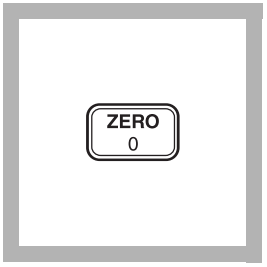
Note: *A diffuser band covers the light path holes on the adapter to give increased performance. The band should NOT be removed.*



4. Fill a Test 'N Tube vial with deionized water. Label this vial as the "blank". Wipe the outside of all the vials with a tissue to remove fingerprints and smudges.



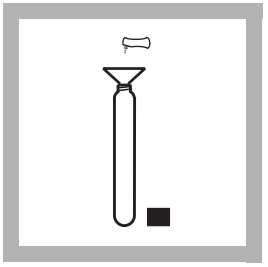
5. Place the blank in the adapter. Tightly cover the vial with the instrument cap.



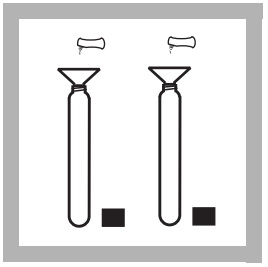
6. Press: **ZERO**

The cursor will move to the right, then the display will show:

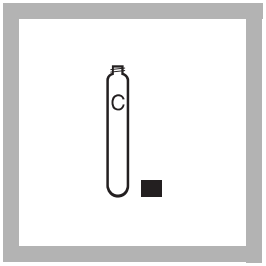
0.000 ABS



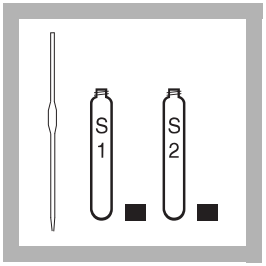
7. Label a vial "control." Open one ToxTrak Reagent Powder Pillow and add the contents to the empty reaction vial.



8. Label each sample or dilution vial clearly. Add the contents of one ToxTrak Reagent Powder Pillow to each labeled vial.

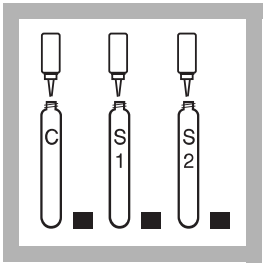


9. Add 5.0 mL of deionized water to the control tube.

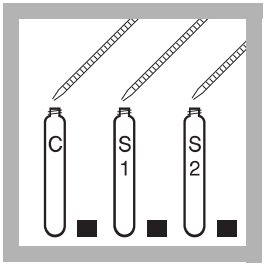


10. Add 5.0 mL of the sample (or dilution) to each sample vial.

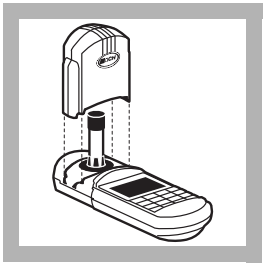
Note: *To determine the approximate threshold level of toxicity for a sample, dilute 1 mL of sample to 10 mL of deionized water and run the test. Continue to make serial 1:10 dilutions until a level is reached which gives a 0% Inhibition in Step 18.*



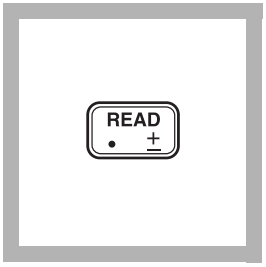
11. Add 2 drops of Accelerator Solution to each vial. Cap and invert to mix.



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

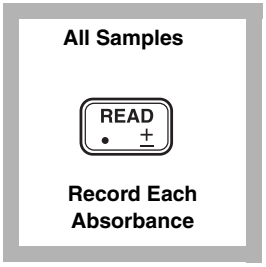


13. Place the control vial in the cell holder. Tightly cover the vial with the instrument cap.

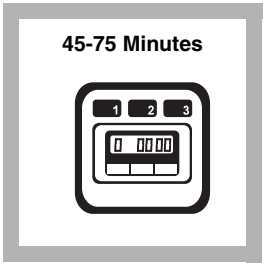


14. Press: **READ**

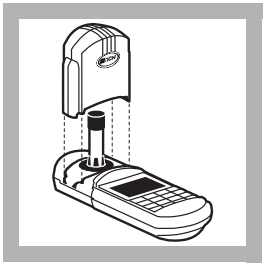
The cursor will move to the right, then the result in ABS will be displayed. Record the absorbance of the "control" vial.



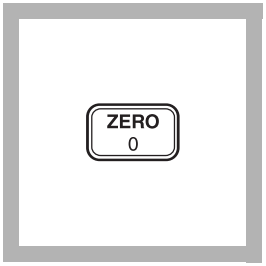
15. Repeat Steps 13-14 for all samples and dilutions. Be sure to record each absorbance.



16. Allow the solutions in the tubes to react until the absorbance of the **control tube** decreases 0.60 ±0.10.
This should take about 45-75 minutes.



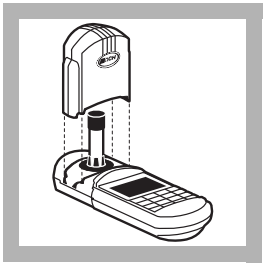
17. After the absorbance of the "control" vial has decreased 0.60 ±0.10 absorbance units, place the blank in the adapter. Tightly cover the vial with the instrument cap.



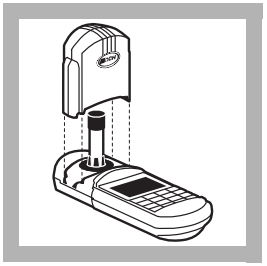
18. Press: **ZERO**

The cursor will move to the right, then the display will show:

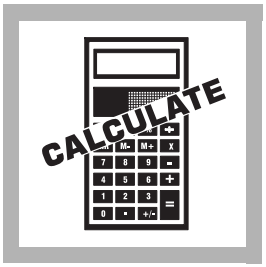
0.000 ABS



19. Place the "control" vial in the cell holder. Tightly cover the vial with the instrument cap. Record the absorbance value of the control.



20. Place each sample or dilution vial in the cell holder. Tightly cover the vial with the instrument cap. Record each absorbance value.



21. Calculate the % Inhibition as follows:

$$\%I = \left[1 - \left(\frac{\Delta Abs\ sample}{\Delta Abs\ control}\right)\right] \times 100$$

ΔA is the initial absorbance value minus the final absorbance value.

See the example following this step.

Note: *Some toxins increase respiration and will give a negative % inhibition on all respiration-based toxicity tests. After repeated testing, samples which always give a % inhibition in Step 21 that is more negative than –10% should be considered toxic.*

Example

The control tube (C) has an initial absorbance of 1.6 and decreases to 1.0 Abs. The sample tube has an initial absorbance of 1.7 and decreases to 1.3 Abs.

$\Delta Abs.\ Sample = 1.7 - 1.3 = 0.4$ $\Delta Abs.\ Control = 1.6 - 1.0 = 0.6$

$$\%I = \left(1 - \left(\frac{0.4}{0.6}\right)\right) \times 100$$

$\%I = 33.3$

Interpreting Results

The Percent Inhibition results obtained are only a relative measurement. They do not represent a true quantitative measurement of toxic concentration. The Percent 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 Percent Inhibition for the dilutions. When the sample is diluted 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 that 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 the table below.

Toxicity Results

Data Points: Percent 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 Percent Inhibition on this and all other respiration-based toxicity tests. After repeated testing, samples that always give a Percent Inhibition that is more negative than –10% should be considered toxic.

Disposal of Test Cultures

Dispose of active bacterial cultures 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 re-use.

Summary of Method

Resazurin is a redox-active dye, which changes from pink to blue when it is reduced. Bacterial respiration occurring in the sample reduces resazurin. If toxic substances are present, they inhibit the rate of resazurin reduction. The sample color is compared to a toxin-free control tube to determine how toxic the sample is to an indigenous culture or a culture of *E. coli*. A chemical accelerant reduces the reaction time of the procedure.

REQUIRED REAGENTS

Description	Cat. No.
ToxTrak Reagent Set (25 tests)	25972-00
Includes: (1) 25607-66, (1) 25608-36, (1) 22777-00, (1) 24092-32	

Description	Quantity Required Per Test	Unit	Cat. No.
Aqua QC–Stiks, Escherichia Coli	1	3 cultures	27063-03
Sodium Thiosulfate Standard Solution	varies	100 mL	24092-32
ToxTrak Reagent Powder Pillows	1 pillow	50/pkg	25607-66
ToxTrak Accelerator Solution	2 drops	15 mL SCDB	25608-36
Tryptic Soy Broth Tubes	1	15/pkg	22777-00
Tube, culture, with cap	1	10/pkg	20962-08
Water, deionized	varies	200 mL	272-29

REQUIRED APPARATUS

Cap, White	1	6/pkg	22411-06
Clippers, to open powder pillows	1	each	936-00
COD/TNT Adapter	1	each	48464-00
Dropper Pipet, 1 mL	varies	10/pkg	21247-20
Forceps, flat square tip	1	each	14537-00
Pipet, volumetric, 5.00 mL, Class A	1	each	14515-37
Pipet Filler, Safety Bulb	1	each	14651-00
Vial, Test ‘N Tube	1	6/pkg	25831-25

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Burner, Alcohol, 60 mL	each	20877-42
Burner, Bunsen	each	21627-00
Germicidal Cloth	50/pkg	24632-00
Incubator, Dri Bath, 25 well, 120/230 V	each	45900-00
Incubator, Dri Bath, 25 well, 120/230 V, with European power cord	each	45900-02
Pipet, Sterile Transfer	15/pkg	22325-12
Timer	each	26305-00

For Technical Assistance, Price and Ordering

In the U.S.A.—Call 800-227-4224

Outside the U.S.A.—Contact the Hach office or distributor serving you.