Toxicity

DOC316.53.01141

ToxTrak™ Method[1, 2] **Method 10017**

0 to 100% inhibition **Test 'N Tube™ Vials**

Scope and application: For drinking water, wastewater and natural waters.

[1] Liu, D., Bull. Environ. Contm. Toxicol. 26, 145-149 (1981)
[2] Environmental Technology Verification ETV Program evaluated, November, 2003

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows adapter and light shield requirements for the instruments that use them.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for test tubes

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800, DR 2700	—	LZV646
DR 1900	9609900 (D[1])	—
DR 900	4846400	Cover supplied with the instrument

[1] The D adapter is not available with all instrument versions.

Before starting

Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.
DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before this test is started.
Do not leave the tubes in the instrument during incubation. Make sure that all samples and control cells have similar conditions of temperature and light during the reaction.
If the samples contain chlorine, add two drops of sodium thiosulfate to each blank and sample before the test is started.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Description	Quantity
Bacterial Count Broth Tube	1 tube
Pipet, transfer, sterile	2
Test 'N Tube, with cap	1
Sodium Thiosulfate	varies
ToxTrak™ Reagent Powder Pillows	2
ToxTrak™ Accelerator Solution	4 drops

Items to collect (continued)

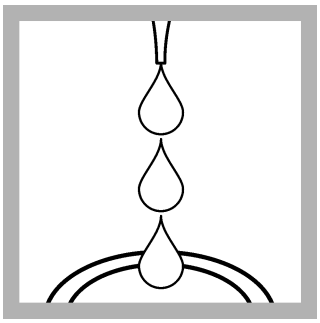
Description	Quantity
Water, deionized	varies
Clippers	1
Incubator	1
Pipet, volumetric, Class A, 5.00 mL and pipet filler	1

Refer to Consumables and replacement items on page 6 for order information.

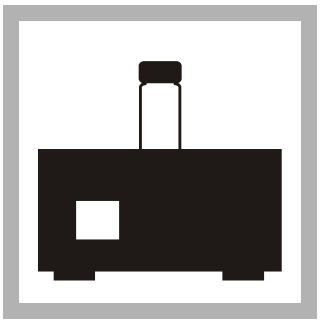
Sample collection

- Collect samples in clean glass or plastic bottles.
- If the sample is drinking water, take the control sample from a reservoir of tap water that is known to be free of toxins if possible.

Inoculum development with indigenous biomass

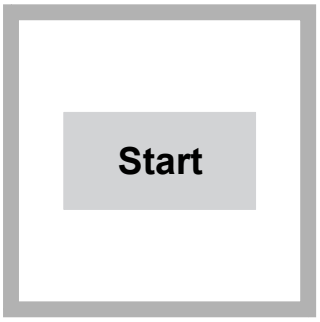


1. Use one of the supplied dropper pipets to add 1.0 mL of source culture (indigenous biomass) to a Total Bacteria Count Broth Tube.
Commercial sources of freeze-dried bacteria may also be used.

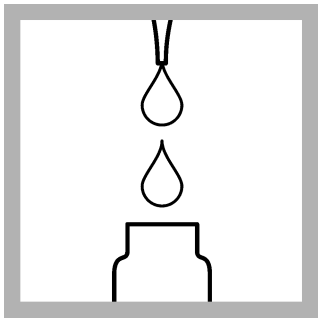


2. Incubate the tube contents at 35 °C (95 °F) until the broth is visibly turbid (approximately 12 hours).
The culture can be kept for several days in the incubator or at room temperature. Use before 72 hours for best results.

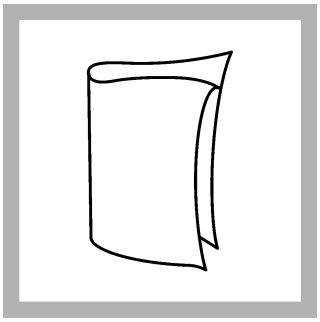
Reaction tube colorimetric procedure



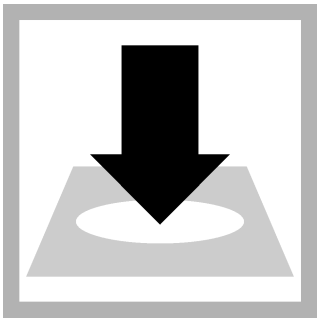
1. Push **Single Wavelength** and enter the wavelength. Refer to Summary of method on page 6 and Instrument-specific information on page 1.



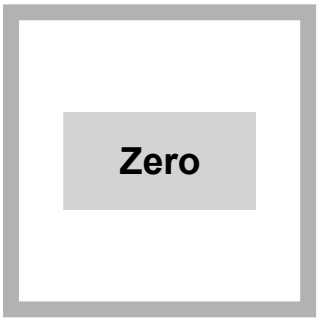
2. Prepare the blank: Fill an empty Test 'N Tube vial to the top of the label with deionized water.



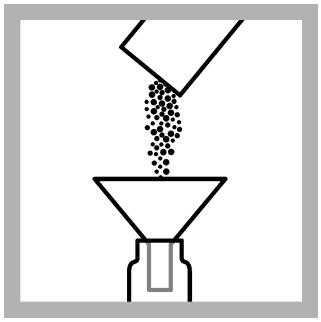
3. Clean the blank sample cell.



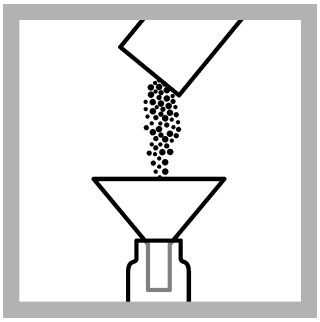
4. Insert the blank into the cell holder.



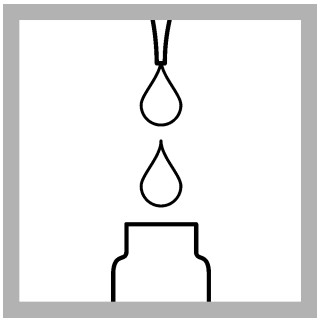
5. Push **ZERO**. The display shows 0.000 Abs.



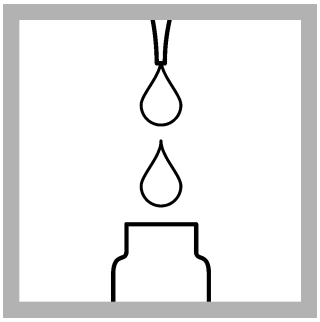
6. Write "control" on one Test 'N Tube vial. Then, open one ToxTrak Reagent Powder Pillow and add the contents to the empty tube.



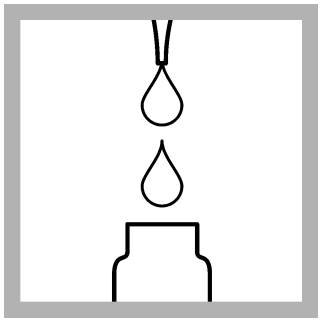
7. For each sample or dilution, write the sample number on each Test 'N Tube vial. Then, open one ToxTrak Reagent Powder Pillow and add the contents to the empty sample vial.



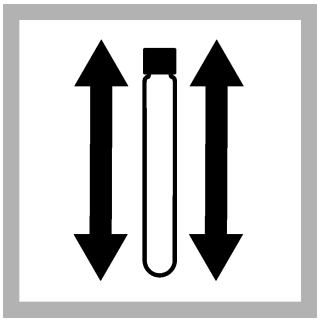
8. Add 5.0 mL of deionized water to the Test 'N Tube vial. Use deionized water that is free of toxicity or another water source that represents baseline toxicity.



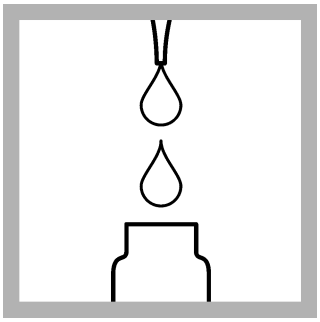
9. Add 5.0 mL of sample (or dilution) to each sample vial. Refer to Interpreting results on page 5 to find the approximate threshold level of toxicity for a sample.



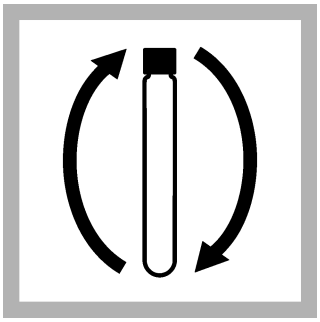
10. Add two drops of Accelerator Solution to each vial.



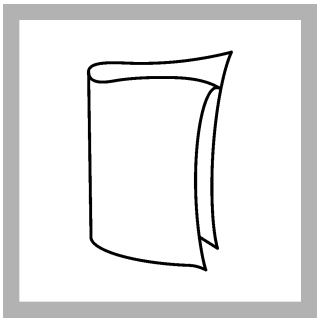
11. Close the tube and shake to mix.
Shake to fully oxygenate the samples, so that the oxygen concentration does not affect the respiration rate.



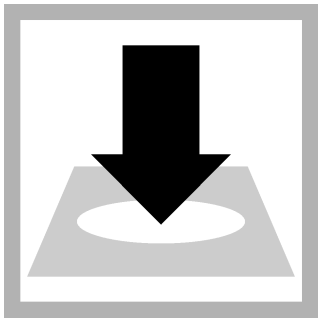
12. Add 0.5 mL of the inoculum (previously prepared) to each tube.



13. Close the tube and invert to mix.



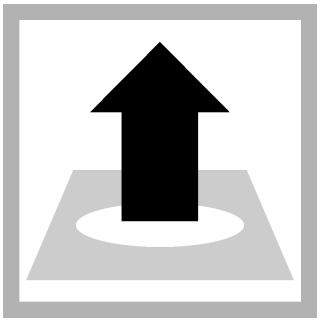
14. Clean the "control" vial.



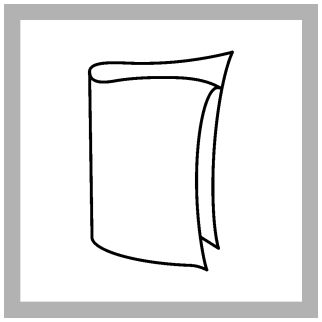
15. Insert the "control" into the cell holder.

16. Push **READ**. Record the absorbance value. Repeat step 14 and 15 for all samples and dilutions. Record all of the absorbance values.

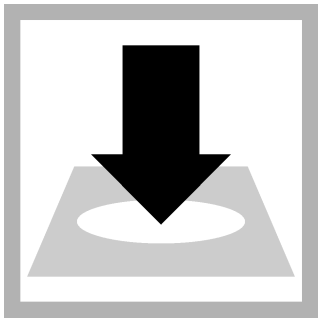
17. Let the solutions in the tubes react until the absorbance of the "control" has decreased by 0.60 (± 0.10) Abs. This takes 45–75 minutes. Invert occasionally.
The reaction time varies according to temperature, age of the culture, bacteria concentrations, etc.



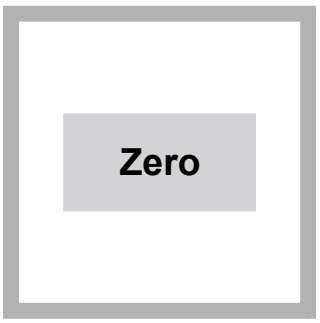
18. Remove the "control" from the cell holder after the absorbance of the control has decreased by 0.60 (± 0.10) Abs.



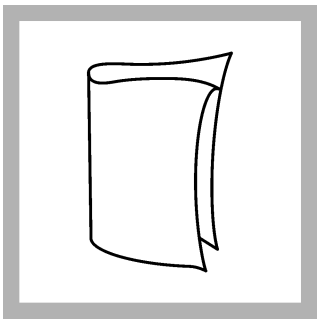
19. Clean the blank sample cell.



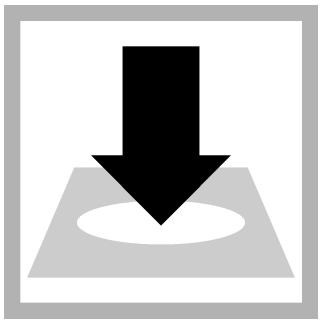
20. Insert the blank into the cell holder.



21. Push **ZERO**. The display shows 0.000 Abs.

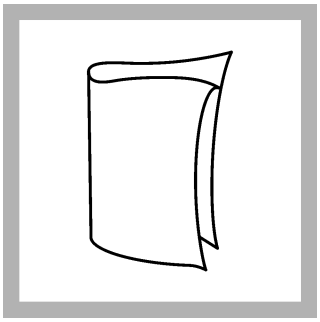


22. Clean the "control" vial.

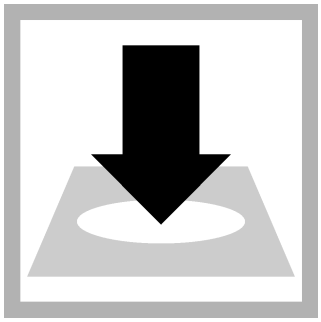


23. Insert the "control" into the cell holder.

24. Push **READ**. Record the absorbance value.

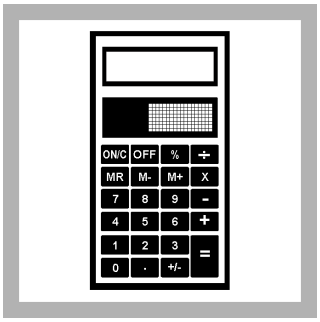


25. Clean each sample or dilution vial.



26. Insert each sample or dilution into the cell holder.

27. Push **Read**. Record all absorbance values.



28. Calculate the % Inhibition. Refer to Calculate the Inhibition on page 5.

Calculate the Inhibition

Calculate the % Inhibition:

$\% I = 1–(\Delta A_{sample}/\Delta A_{control}) \times 100$

where: Δ A = Initial absorbance value–Final absorbance value

Example:

Absorbance of control: initial = 1.500 abs, final = 0.900 abs; $\Delta A_{control} = 0.600$

Absorbance of sample: initial = 1.700 abs, final = 1.300 abs; $\Delta A_{sample} = 0.400$

$\% I = 1–(0.400/0.600) \times 100 = 33\%$

Interpreting results

The results as percent inhibition (% I) are a relative measurement. The results 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.

Results below 10% are not reliable, but can be used to make an estimate of toxicity when the results are consistent. If a sample shows less than 10% inhibition, repeat the test several times. Look at the series of data points to find the likelihood of toxicity. Refer to Table 2.

Some toxins will increase respiration and 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.

Table 2 Interpreting results that are less than 10% inhibition

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

Lowest observable effect concentration (LOEC)

Due to the many variables involved in the test, the limit of detection is approximately 10% inhibition. This correlates to the Lowest Observable Effect Concentration (LOEC).

No observed effect concentration (NOEC)

To determine the minimum inhibition concentration of a toxin:

1. Dilute 1 mL of sample to 10 mL with deionized water.
2. Run the test and find the percent inhibition for the dilution.
3. Dilute 1 mL of the sample dilution from step 1 to 10 mL with deionized water.
4. Run the test and find the percent inhibition for the dilution.
5. Continue to make serial 1:10 dilutions of the sample (1:10, 1:100, 1:1000, etc.) until a level is reached that gives 0% inhibition in the final calculation.

When 0% inhibition is found, the dilution represents the approximate threshold level of toxicity for a sample. This is the No Observed Effect Concentration (NOEC).

Disposal of test cultures

Use one of the methods that follow to dispose of active bacterial cultures:

- 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 reused.
- Sterilize test containers with 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. The measurement wavelength is 603 nm for spectrophotometers or 610 nm for colorimeters.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
Water, deionized	varies	500 mL	27249
ToxTrak™ Reagent Set	1	25/set	2597200
Includes:			
Media Set, Total Bacteria Count Tubes	1	15/pkg	2277700
Pipet, transfer, sterile	1	15/pkg	2232512
Sodium Thiosulfate Standard Solution, 0.0246 N	varies	100 mL	2409232
ToxTrak™ Reagent Powder Pillows	2	50/pkg	2560766
ToxTrak™ Accelerator Solution	4 drops	15 mL SCDB	2560836
Tubes, glass, 16-mm x 100-mm	1	6/pkg	2275806
Caps, white, Teflon lining, for 16-mm vials	2	6/pkg	2241106

Required apparatus

Description	Quantity/test	Unit	Item no.
Clippers	1	each	93600
Dropper, measuring, 0.5-mL and 1.0-mL plastic	2	20/pkg	2124720
Forceps, flat square tip	1	each	1453700
Incubator, Dri-Bath, 12-well, 120 VAC	1	each	2281400
Pipet, volumetric, Class A, 5.00-mL	1	each	1451537
Pipet filler, safety bulb	1	each	1465100

Optional reagents and apparatus

Description	Unit	Item no.
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips for TenSette® Pipet, 1.0–10.0 mL	50/pkg	2199796
Test tube rack, stainless steel	each	1864100
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Pipet tips for TenSette® Pipet, 1.0–10.0 mL	250/pkg	2199725
BOD seed (polyseed)	50/pkg	2918700
Laboratory pen, permanent marker	each	2092000

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