DeltaTox
User's Manual

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INTRODUCTION

Description

The DeltaTox System is a simple, rapid and portable test method for toxicity screening and monitoring of environmental samples. The System includes a highly sensitive analyzer (luminometer), freeze-dried bacterial reagent, and test control and reconstitution solutions. The built-in software of the analyzer is menu driven for ease of use and operation.

The System is designed to operate and test samples at a wide range (10ºC - 28ºC) of ambient temperatures. This allows the flexibility of field testing under variable environmental conditions. The analyzer reads and records a wide dynamic range of light emitted by the test reagent.

Operational Background

The DeltaTox System is capable of performing the dual functions of toxicity testing and ATP measurement. Both of these functions measure light, but from different biological processes. The TOX (Q-Tox or B-Tox) function reads light produced by luminescent bacteria (*Vibrio fischeri*) after exposure to a test sample, and compares it to the light output of a control (reagent blank). The degree of percent light loss (an indication of metabolic inhibition in the test organisms) indicates the relative toxicity of the sample. The ATP function measures light produced by an interaction between adenosine triphosphate (ATP) and Luciferin/Luciferase reagents.

The operation of the analyzer is managed with three function read modes which are selected with the MODE key. In each mode, pressing the START key creates a data file for the new set of data about to be generated.

The results are stored internally and do not require a battery to be maintained. The data can be viewed via the LCD with the CYCLE function. Also, the data can be downloaded to an ASCII text file using a standard telecommunications, or data transfer, software program.

The instrument is designed for field use and will operate continuously on a fresh set of alkaline batteries for 10 to 12 working hours. The unit will store approximately 600 reads in memory.

The DeltaTox System offers a wide dynamic range of light measurement (from 0 to approximately 120,000,000 photon counts). All test range sensitivities are automatically selected and calibrated for high accuracy readings. Warning messages alert the user to over or under ambient temperature operating conditions, motor and optical switch failures, count overflow, and low battery conditions.

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SYSTEM COMPONENTS

The DeltaTox System includes the following components:

- DeltaTox Carrying Case
- DeltaTox Analyzer (with AC Power adapter)
- Microtox Solo Reagent
- Microtox Diluent
- Microtox Osmotic Adjusting Solution
- Disposable Glass Cuvettes
- Pipettors: 10 µl pipettor and tips, 100-1000 µl pipettor and tips
- Cuvette Rack
- DeltaTox User's Manual

Please unpack the system and check that all components are included and that all are in good condition. Contact Strategic Diagnostics, Inc. Customer Service or your local distributor to report any missing, broken or defective items.

NOTE: Upon receipt of the system, immediately locate and store the SOLO (or other) Reagent in a freezer at -20ºC to -25ºC.

For operating the DeltaTox Analyzer from an AC outlet, connect the AC Power Adapter to the Adapter Port of the DeltaTox (see Figure 1).

For field use, please install four alkaline "C" cell batteries in the Battery Holder (see Figure 1) of the DeltaTox.

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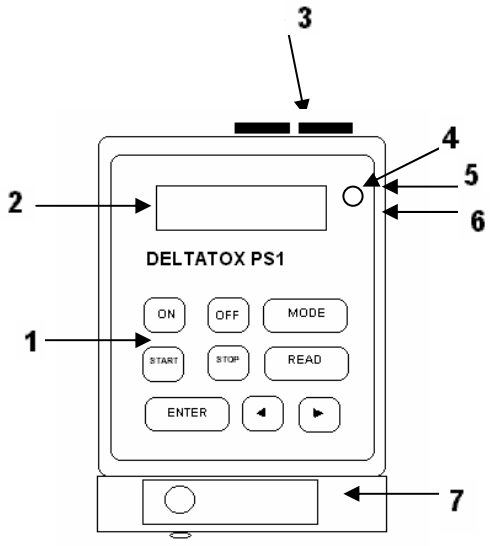
OPERATION

Features:



1. **Keypad**
   A standard 9 key membrane keypad, which is used for all operational commands

2. **LCD Display**
   The LCD is a 40 character (2 lines of 20 characters each) display device

3. **Battery Holders**
   Panel mount "C" type batter holders. Each batter compartment holds two batteries. The ends of the holders unscrew for batter replacement

4. **Alert LED**
   The Alert LED lights (and an alarm sounds) to prompt the operator to begin taking readings at the end of countdown periods, indicates motor and switch failures, or signify overrange conditions.

5. **RS-232 Serial Port**
   The I/O port located on the right side near the back of the unit is used to download stored data records.

6. **AC Power Adapter Port**
   The AC to DC power supply input is located on the right side near the back of the instrument.

7. Read Assembly

The Read Assembly contains the following components:

a. Photomultiplier Tube (PMT)
   This is the light sensing device. The PMT counts photon events from light sources placed in front of its input window.

b. Filter Drive System
   The Filter Drive System automatically inserts a neutral density filter which extends the dynamic range capability of the analyzer

c. Temperature Sensor
   The Temperature Sensor is mounted in the Read Assembly adjacent to the cuvette well, and is used to monitor ambient temperature. The current temperature is displayed and recorded by the analyzer. The instrument will only operate within the specified 10ºC - 28ºC range.

d. Safety Shutter
   The Safety Shutter protects the PMT read window from ambient light whenever the Sample Chamber lid and latch are in the open position.

e. Sample Chamber
   The well into which the cuvettes containing either the control or sample are placed for taking a light output readings.

f. Lid and Latch
   The lid is used to cover the Sample Chamber when taking light readings. The latch is used for locking the lid in the closed position.

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TEST SAMPLES AND TESTING MATERIAL

Sample Types

A wide variety of environmental samples may be tested with the DeltaTox, including raw water, drinking water, wastewater, industrial discharges, influent and effluent, spills, septage and extracts of soils and sediments.

Sample Collection

Ideally, samples should be collected in new borosilicate glass screw cap containers (30 to 50 ml) with Teflon® lined caps (polycarbonate and polypropylene containers are also suitable). Fill the container completely to the top with sample, leaving no airspace. Completely filling the container helps keep volatile material in solution.

Sample Storage

Samples should be tested as soon as possible after collection to prevent unpredictable changes. If testing is delayed, store samples at normal refrigerator temperature (2ºC to 8ºC). Samples can change with time, so testing the samples within 2-4 hours after collection is best, although this may not always be possible. It is suggested to test samples within 72 hours after collection.

Sample Preparation

Most samples will not require any special preparation for analytical testing. However, certain samples may require special preparation due to specific testing requirements as outlined below:

1. **Turbid Samples**
   Samples which are turbid or contain particulate matter that will not settle may require clarification due to certain regulatory and/or standard operating procedure requirements. The clarification of such samples can be accomplished by centrifugation at an adequate speed and time to remove the turbidity and/or particulate material. It should be noted that the sample turbidity may cause a non-specific increase or decrease in light output and this process should only be undertaken if toxicity due to turbidity is NOT desired.

2. **Colored Samples**
   Samples which are highly colored (specifically red, brown or black) may interfere with the test results by absorbing light. Such samples should be diluted (i.e. 25% or 50%) with distilled or deionized water before testing.

3. **Samples containing chlorine**
   Samples which contain chlorine as a result of chlorination will interfere with the test result by affecting the viability of the bacterial reagent. Such samples should be dechlorinated using a sodium thiosulfate solution. See the “Preparation of Sodium Thiosulfate Stock Solution” and “Dechlorination with Sodium Thiosulfate” in the Appendix.

4. **Sample pH**
   The sample pH may affect test results in an unpredictable manner. Ideally, samples should be tested at their native pH (without pH adjustment), however the test reagent

exhibits optimum light output within the range of pH 6.0 to 8.0. A sample pH outside of this range can affect the toxicity measurement, so the pH may be adjusted on samples which are below 6.0 and above 8.0 and as stipulated by certain regulatory and/or standard operating procedure requirements. The adjustment of sample pH should be performed as described in the Appendix (see “Adjusting pH of Sample”). pH modification may impact test relevance and sample integrity.

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Reagent and Supplies

SOLO Reagent
The freeze dried reagent should be stored at -20°C to -25°C to maintain activity.

The freeze dried reagent is stable for 4 weeks when stored at 4°C. It is recommended that if it is necessary to transport the reagent, a thermally insulated container and frozen ice packs are used. The freeze dried reagent is stable for 24 hours at 22°C. However, it is recommended that the reagent should, whenever possible, be kept at -20°C or 4°C until required for use.

Once the reagent has been reconstituted, it is recommended that it is used within 30 minutes. The recommended reconstitution time is 15 minutes. It is possible that the sensitivity of the reagent to some samples may change if the reconstitution time is extended beyond 30 minutes.

Microtox Diluent
Microtox Diluent is used to reconstitute the reagent, dilute samples and act as a non-toxic control sample. Its shelf life is one year when stored at room temperature.

Microtox Osmotic Adjusting Solution
Microtox Osmotic Adjusting Solution (OAS) is a specially prepared non-toxic 22% sodium chloride solution. It is used to bring the salinity of the samples to approximately 2%, by adding one part OAS to 10 parts sample.

The OAS has a shelf life of one year when stored at room temperature. (Do not make up OAS yourself or use substitutes.)

Cuvettes
Glass cuvettes are used to contain samples, controls, and reagent during testing. Do not reuse cuvettes.

Cuvette Rack
The cuvette rack is designed for holding the cuvettes in order to set up and conduct a test. The rack can hold up to 50 cuvettes. The rack contains 5 rows, labeled A to F from top to bottom on the left side of the rack, and each row contains ten (10) wells labeled from 1 to 10 (from left to right).

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TESTING CONSIDERATIONS

Time and Temperature

Different chemicals affect living organisms at different rates, reflecting differences in mechanism of action. For some classes of chemicals, the effect on light output is complete in 5

minutes. For other classes of chemicals, 15 minute data may be more reliable. The DeltaTox has a default setting of 5 minutes.

The System operates satisfactorily at a wide range (10ºC - 28ºC) of ambient temperatures which allows the flexibility of testing samples under variable environmental conditions. With no operational temperature control and associated variable light output by the test reagent, some variation may be observed in data and test results obtained at different temperatures.

Reagent Blank (Control)

A reagent blank (control) is required for each toxicity test and is run concurrently with the sample. Light levels normally change with time for reasons other than the bioreactivity of the test sample. All response of light changes in the sample are compared to that of the reagent blank (control).

Interferences

Samples which contain large amounts of particulate matter will interfere with the test. Diluting or centrifuging the sample may be required. Strongly colored samples (particularly red or brown) will also interfere with test results and may require primary dilution before testing.

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TEST MODE AND ANALYTICAL PROCEDURES

TOX Mode Testing

The DeltaTox System provides two standardized testing procedures for acute toxicity measurement and assessment. The first is the Q-Tox Procedure which has been developed for quick toxicity screening. This test procedure is preferred when several samples must be tested quickly or when only a rough estimate of toxicity level is desired. The second test procedure is the B-Tox Procedure. It is a basic toxicity screening test which is preferred when a more precise result is desired. The B-Tox Procedure involves taking zero time readings of the rehydrated DeltaTox test reagent prior to sample introduction, whereas the Q-Tox Procedure does not involve this step.

No special sample preparation, such as osmotic adjustment of the sample, is required by either of the two testing procedures. All test solutions, samples and analyzer must be at ambient temperature (10ºC - 28ºC) at the time of conducting tests. The analyzer will not function reliably outside of this temperature range.

2% B-Tox Test Procedure for High Toxicity Samples

This method is used for samples where "high" toxicity is expected (i.e. samples such as septage and waste water treatment plant influent).

Analyzer Setup:

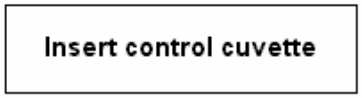
1. Place the analyzer on a level and dry surface
2. Power on the unit by pressing the ON key. The analyzer will perform a 1 minute self-test.
3. If the 1 minute self-test is successful, the Default Power-Up screen (Default screen) will appear. Verify that the analyzer temperature (on the display) is within a range of 10ºC - 28ºC.
4. Press the MODE key until "B-Tox" appears
5. Set the DeltaTox for a 5-minute incubation time.

Test Procedure:

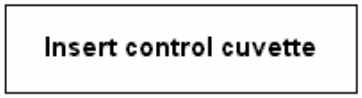
6. Place cuvettes in wells A1 and A2 of the cuvette rack
7. Reconstitute a vial of Microtox SOLO Reagent using 2.5 mL (2500 µL) of Microtox Diluent.
8. Pour the reconstituted Reagent into a cuvette
9. Mix reagent 3-4 times using the 500 µL pipettor
10. Add 1000 µL of reconstituted Reagent into the two (2) cuvettes in A1 and A2.
11. Wait 15 minutes.

12. **Without** a cuvette in the Sample Chamber (make sure that the lid is down and the latch is completely closed), press the START key. The instrument will display in sequence: "Close lid and latch," "Waiting for PMT to warm up," "Reading dark

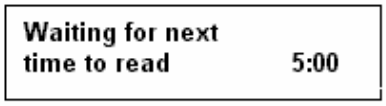
current. Please wait." During this time the instrument creates and opens a record. The screen then displays:



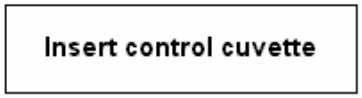
13. Place the control cuvette (A1) into the Sample Chamber. Close the lid and the latch. Press the READ key to take a reading of the control cuvette (A1).
14. Remove the A1 cuvette from the DeltaTox and place it back into the cuvette rack.



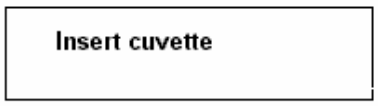
15. Place the A2 control cuvette into the Sample Chamber. Close the lid and latch. Press the READ key.
16. Press the STOP key, remove the A2 cuvette from the DeltaTox and place it back into the cuvette rack.



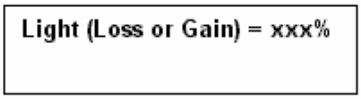
17. Immediately add 10 μL of Sample (at room temperature) to cuvette A2.
18. Mix contents of A2 by swirling.
19. Following the 5 minute countdown, an alarm will sound and the following display will appear:



20. Insert the A1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
21. Remove the A1 cuvette from the DeltaTox and place it back into the cuvette rack.



22. Insert the A2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key
23. The DeltaTox Analyzer will then automatically display the % Light Loss or Gain.



24. Press the STOP key to return to the Default screen

Multiple Samples
Should there be a need to analyze multiple samples, the above procedure can be used with the following additional steps:

25. Place cuvettes in the cuvette rack. The number of samples to be tested should equal the number of cuvettes (plus one for the control)
26. Add 1000 µL reconstituted reagent to each cuvette
27. Wait 15 minutes
28. Take the zero time readings
29. Add 10 µL of each sample to its corresponding cuvette
30. Following the 5 minute waiting period, insert the cuvettes for the second reading of each
31. When the last cuvette is read, press the STOP key to stop taking readings and terminate a testing sequence. The display will show, "Tox Readings Done," and the result of the last cuvette read.
32. Press the STOP key again to return to the Default screen.

Results Review
33. The results are now available for review in chronological order of sample testing
34. To review the results:

- Press the ENTER key and use the Right Arrow key (>) to scroll to "Dat."
- Dat. ENTER
- Cycle, ENTER
- Scroll to "B-Tox," ENTER

The record header for the first record created (Record #001) will appear and the following will be displayed:

Pressing the Right Arrow key (>) will allow you to scroll through each "Read" (sample) of each "Record." If the "Record" that you need to review is not in view, continue pressing the Right Arrow key (>) until it comes into view. When the last "Read" of the last "Record" is reached, the message "No more records" will appear. To return to the Default screen from any "Read" of any "Record," press the ENTER key

Analyzer Shut-Off
If the analyzer is not used for any more testing or data review, turn off the analyzer by pressing the OFF key. *(NOTE: After 30 minutes of non-use, the unit shuts off automatically.)*

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45% B-Tox Test Procedure for Medium Toxicity Samples

This procedure should be used with samples where a "medium" level of toxicity is expected (i.e. storm water samples).

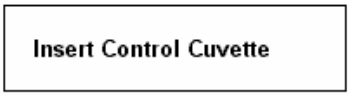
Analyzer Set-Up

1. Place the analyzer on a level and dry surface.
2. Power on the unit by pressing the ON key. The analyzer will perform a 1 minute self-test.
3. If the 1 minute self-test is successful, the Default Power-Up screen (Default screen) will appear. Verify that the analyzer temperature (on the display) is within a range of 10°C - 28°C.
4. If the display does not indicate the desired mode (B-Tox), press the MODE key until "B-Tox" appears.
5. Set the DeltaTox for a 5-minute incubation time.

Test Procedure

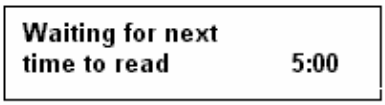
6. Place cuvettes in wells A1, A2, B1 and B2 of the cuvette rack.
7. Add 1000 µL Microtox Diluent to cuvette A1
8. Add 1000 µL of Sample to cuvette A2
9. Add 100 µL of Microtox OAS to cuvette A2 and mix using pipeettor
10. Reconsititute a vial of Microtox SOLO Reagent using 1.5 mL (1,500 µL) of Microtox Diluent
11. Pour the reconstituted Reagent into a cuvette
12. Mix reagent 3-4 times using the 500 µL pipetor
13. Add 500 µL reconstituted Reagent into two (2) cuvettes B1 and B2.
14. Wait 15 minutes

15. **Without** a cuvette in the Sample Chamber (make sure that the lid is down and the latch is completely closed), press the START key. The instrument will display in sequence: "Close lid, and latch," "Waiting for PMT to warm up," "Reading dark current. Please wait." During this time the instrument creates and opens a record.
16. The instrument will then prompt the operator to "Insert control cuvette" in preparation for the first set of readings (zero time readings).

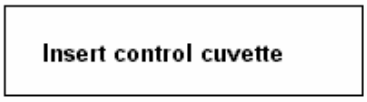


17. Insert the B1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
18. Remove B1 from the DeltaTox and place it back into the cuvette rack.

19. Insert B2 (Sample) cuvette into the Sample Chamber. Close the lid and latch. Press the READ key.
20. Press the STOP key, remove B2 from the DeltaTox and place it back into the cuvette rack.

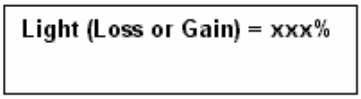


21. Immediately add 500 µL from A1 to B1 (Control at room temperature)
22. Transfer 500 µL from A2 to B2 (Sample at room temperature)
23. Mix contents of B1 and B2 by swirling.
24. After the timer finishes, the alarm will sound and the alert LED will light indicating the instrument is ready to take the second set of readings.



25. Insert B1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
26. Remove B1 from the DeltaTox and place it back into the cuvette rack.

27. Insert B2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
28. The DeltaTox Analyzer will then automatically display the % Light Loss or Gain.



29. Press the STOP key to return to the Default screen

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81.9% B-Tox Test Procedure for Low Toxicity Samples

This procedure should be used with samples where a "low" level of toxicity is expected (i.e. waste water treatment effluent samples).

Analyzer Set-Up

1. Place the analyzer on a level and dry surface.
2. Power on the unit by pressing the ON key. The analyzer will perform a 1 minute self-test.
3. If the 1 minute self-test is successful, the Default Power-Up screen (Default screen) will appear. Verify that the analyzer temperature (on the display) is within a range of 10°C - 28°C.
4. If the display does not indicate the desired mode (B-Tox), press the MODE key until B-Tox appears.
5. Set the DeltaTox for a 5-minute incubation time.

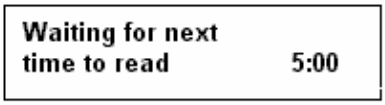
Test Procedure

6. Place cuvettes in wells A1,A2, B1 and B2 of the cuvette rack.
7. Add 1000 µL Microtox Diluent to cuvette A1
8. Add 1000 µL Sample to cuvette A2
9. Add 100 µL Microtox OAS to cuvette A2 and mix using the pipettor
10. Reconstitute a vial of Microtox SOLO Reagent using 300 µL of Microtox Diluent
11. Mix reagent 3-4 times using the 100 µL pipettor
12. Add 100 µL reconstituted Reagent into two (2) cuvettes B1 and B2.
13. Wait 15 minutes

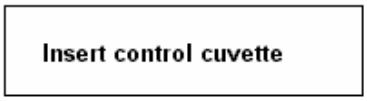
14. **Without** a cuvette in the Sample Chamber (make sure that the lid is down and the latch is completely closed), press the START key. The instrument will display in sequence: "Close lid, and latch," "Waiting for PMT to warm up," "Reading dark current. Please wait." During this time the instrument creates and opens a record.
15. The instrument will then prompt the operator to "Insert control cuvette" in preparation for the first set of readings (zero time readings).

16. Insert the B1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
17. Remove the B1 cuvette from the DeltaTox and place it back into the cuvette rack.

18. Insert B2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
19. Press the STOP key, remove B2 cuvette from the DeltaTox and place it back into the cuvette rack. The DeltaTox begins a 5 minute countdown .

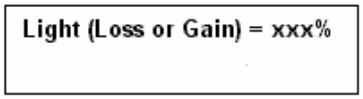


20. Immediately transfer 900 µL from A1 to B1 (Control at room temperature)
21. Transfer 900 µL from A2 to B2 (Sample at room temperature)
22. Mix contents of B1 and B2 by swirling.
23. When the countdown is completed, an alarm will sound and the DeltaTox will prompt the user:



24. Insert B1 (Control cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
25. Remove the B1 cuvette from the DeltaTox and place it back into the cuvette rack.

26. Insert B2 (Sample cuvette) into the Sample Chamber. Close the lid and latch. Press the READ key.
27. The DeltaTox Analyzer will then automatically display the % Light Loss or Gain.



28. Press the STOP key to return to the Default screen

Analyzer Shut-Off

If the analyzer is not used for any more testing or data review, turn off the analyzer by pressing the OFF key. *(NOTE: After 30 minutes of non-use, the unit shuts off automatically.)*

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ATP Mode Testing

The DeltaTox Analyzer is capable of ATP (adenosine triphosphate) measurements. The measurement is based on a bioluminescent method and is performed at ambient temperature. Serial dilutions are made of the sample and exposed to luciferin and luciferase (substrate and enzyme preparation or mixture). Light measurements (photon counts) of the sample are taken with the DeltaTox Analyzer and compared to a standard curve with a resulting amount of ATP concentration. A typical standard curve based on photon counts and ATP concentrations is shown in the Appendix. From this curve, an estimation can be made of the amount of ATP total (living) biomass in the sample. In general, the ATP level is a good indicator of total (living) biomass.

Strategic Diagnostics does not provide an ATP test reagent. A commercial bioluminescent test kit and standardized ATP preparation can be used with the DeltaTox system to perform ATP measurements.

Analyzer Setup:

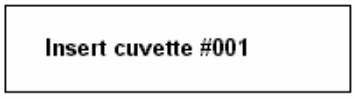
1. Place the analyzer on a level and dry surface
2. Power on the unit by pressing the ON key. The analyzer will perform a 1 minute self-test.
3. If the 1 minute self-test is successful, the Default Power-Up screen (Default screen) will appear. Verify that the analyzer temperature (on the display) is within a range of 10°C - 28°C

   NOTE: If the self-test fails, please refer to the Troubleshooting Section of this manual.

4. If the display does not indicate the desired mode (ATP), press the MODE key until ATP appears.

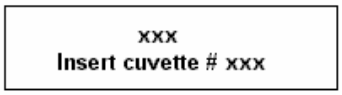
Test Procedure – ATP Standard Curve

5. Follow the instructions of the commercial ATP bioluminescent test kit that you are using for preparing dilutions of the ATP standard. Prepare all dilutions of the ATP standard in the cuvettes for use with the DeltaTox Analyzer.
6. **Without** a cuvette in the Sample Chamber (make sure the lid is down and the latch is completely closed), press the START key. The instrument will display in sequence: "Close lid and latch," "Waiting for PMT to warm up," "Reading dark current. Please wait." During this time the instrument creates and opens a record.
7. Read the ATP standard curve dilutions in their appropriate order as they are ready (low concentrations to high concentrations)
8. The instrument will prompt the operator to insert the first (dilution) cuvette into the Sample Chamber. Close the lid and the latch. Press the READ key to take a reading on the cuvettes.



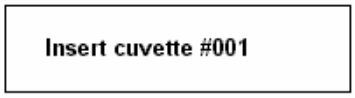
9. The analyzer will them prompt to read the next cuvette. Read all remaining ATP standard curve dilution cuvettes in similar fashion as was done in step 8. As each dilution cuvette is prompted for reading, the photon count **for the previous cuvette** will be displayed.

10. When the last dilution cuvette is read, press the STOP key to end taking readings and terminate a testing sequence. Pressing the STOP key returns to the Default screen. To review results, proceed to Step 17

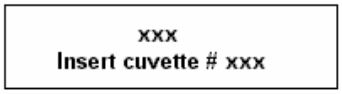


Test Procedure – ATP Sample Analysis

11. Follow the instructions on the commercial ATP bioluminescent test kit (that you are using) for preparing all samples. Prepare all samples in the cuvettes for use with the DeltaTox Analyzer.
12. **Without** a cuvette in the Sample Chamber (make sure the lid is down and the latch is completely closed), press the START key. The instrument will display in sequence: "Close lid and latch," "Waiting for PMT to warm up," "Reading dark current. Please wait." During this time the instrument creates and opens a record.
13. Read the samples in their respective order as they are ready.
14. The instrument will prompt the operator to insert the first sample cuvette (#001) into the Sample Chamber. Close the lid and the latch. Press the READ key to take a reading on the cuvette.



15. The analyzer will prompt to read the next cuvette. Read all remaining samples in similar fashion as was done in Step 14. As each sample cuvette is prompted for reading, the photon count for the previous cuvette just read will be displayed.

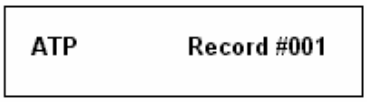


16. When the last sample cuvette is read, press the STOP key to end taking readings and terminate a testing sequence. Pressing the STOP key returns to the Default screen. To review results, proceed to Step 17.

Results Review:
17. To review the results:

- Press the ENTER key and use the Right Arrow key (>) to scroll to "Dat."
- Dat, ENTER
- Cycle, ENTER
- Scroll to "ATP," ENTER

The record header for the first record created (Record #001) will appear and following will be displayed:



Pressing the Right Arrow key (>) will allow you to scroll through each "Read" (sample) of each "Record." If the "Record" that you need to review is not in view, continue pressing the Right Arrow key (>) until it comes into view. When the last "Read" of the last "Record" is reached, the message "No more records" will appear. To return to the Default screen from any "Read" of any "Record," press the ENTER key.

Analyzer Shut-Off
When the analyzer will no be used for any more testing or data review, turn off the analyzer by pressing the OFF key. *(NOTE: After 30 minutes of non-use, the unit shuts off automatically.)*

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DATA DOWNLOAD PROCEDURES

In order to download data from the DeltaTox Analyzer to a computer, the following items are needed:

- A computer running either Windows® or Windows 95.
- A 9 pin male to 9 pin female serial cable

To Download To A Text File with Windows 95

1. Connect the serial cable from the DeltaTox Analyzer to the computer
2. Power on both the DeltaTox and the computer
3. At the Windows 95 main screen load HyperTerminal by choosing the following: "Start," "Programs," "Accessories," "Hyperterminal"

4. When the HyperTerminal folder appears, click on the **Hypertrm** icon
5. HyperTerminal will prompt you for a **New Connection** name. Enter in a name and choose an icon. Click **OK**.
6. The Phone Number menu screen appears. Click on the **Connection Using** box. The box contents will list various parts and devices which can be selected for the configuration of the DeltaTox Analyzer.

   NOTE: The cable provided with the DeltaTox Analyzer is intended for use with a standard 9 pin serial communications port. In most instances COM1 will be the name of the 9 pin port at the back of your computer. If you know your system setup to vary from this configuration, select the correct settings.

7. Choosing **Direct to COM1** will cause the COM PORT properties screen to open. The parameters should be set to the following:

Bits per second:	9600
Data bits:	8
Parity:	none
Hardware:	Flow control

   After selecting these settings, click **OK**.
8. Save these settings with the **SAVE** command from the **FILE** pull-down menu.

   NOTE: Once all of the settings have been selected and saved, the operator only has to click the icon created in Step 5 to ready the HyperTerminal program for data downloading each time the HyperTerminal program is started.

9. Choose the **Transfer** pull-down menu and select **Capture text**.
10. Type in the path and name of the file which you wish the data text file to have and click **OK** (i.e., C:\Program Files\Accessories\HyperTeminal\Capture.txt.)
11. Type in the command "**.Show**" and press the ENTER key. This command will be echoed on the computer screen followed by all data stored in the DeltaTox Analyzer.
12. After the data has stopped downloading (the screen image is no longer scrolling), then return to the **Transfer** pull-down menu and select **Capture text**. This action will open another box. Choose **Stop**. The HyperTerminal program can now be exited.

    NOTE: The text file created in Step 10 is a standard ASCII text file which can be viewed and edited in any standard ASCII text editor.

To Download to a Text File with Windows 3.1 or 3.11

1. Connect the serial cable from the DeltaTox Analyzer to the computer
2. Power on both the DeltaTox Analyzer and computer
3. At the Windows main screen load into Terminal by choosing the following: "Accessories" and the "Terminal"

4. At the Terminal program, choose the **Settings** pull-down menu. Then select **Communications**.

   NOTE: The cable provided with the DeltaTox Analyzer is intended for use with a standard 9 pin serial communications port. In most instances COM1 will be the name of the 9 pin port at the back of your computer. If you know your system setup to vary from this configuration, select the correct settings.

5. Choose **COM1** at the Connector choice box.
6. The communications parameters should set to the following:

Bits per second:	9600
Data bits:	8
Parity:	none
Hardware:	Flow control

7. Select the **Transfer** pull-down menu and choose **Receive Text File**.
8. Type in the path and name of the file which you wish the data file to have. Add a "**.txt**" extension to the end of the file name as Windows does not always do it automatically. If the "**.txt**" extension is not added, the error message "Cannot create specific file" will appear. Then click **OK**.
9. Type in the command "**.Show**" and press the ENTER key. This command will be echoed on the computer screen followed by all data stored in the DeltaTox Analyzer.
10. After the data has stopped downloading (the screen image is no longer scrolling, then return.

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DELTATOX ANALYZER DATA DOWNLOAD

Example of Downloaded Data:

ATP, 001, 002, 01-01-1993, 19.5	*(Record Header Line)*
ATP, 001, 001, 12:02:52, Photon Count = 0	*(Test Result Line, Sample #1)*
ATP, 001, 002, 12:03:52, Photon Count = 16	*(Test Result Line, Sample #2)*
B-Tox, 001, 002, 01-01-1996, 22.3	*(Record Header Line)*
B-Tox, 001, 001, 03:05:44, Light Loss = 63%	*(Test Result Line, Sample #1)*
B-Tox, 001, 002, 03:06:08, Light Loss = 30%	*(Test Result Line, Sample #2)*
Q-Tox, 001, 003, 01-01-1996, 22.6	*(Record Header Line)*
Q-Tox, 001, 001, 03:25:05, Light Gain = 27%	*(Test Result Line, Sample #1)*
Q-Tox, 001, 002, 03:25:13, Light Loss = 35%	*(Test Result Line, Sample #2)*
Q-Tox, 001, 003, 03:25:20, Light Gain = 14%	*(Test Result Line, Sample #3)*

Explanatory Information:

Record Header Line
The components of the Record Header Line reading from left to right are:

1. Test Mode (i.e., ATP, B-Tox or Q-Tox)
2. Record Number (for example, the Q-Tox results above are from Record #001)
3. Read Number (This Read number is the last reading taken during the test. In the case of the Q-Tox record above, the Read number is 003 since the last reading taken was Read #003 (indicating that 3 samples were analyzed)
4. Date (The date the Record was created. For the Q-Tox record above, it was created on 01/01/1996)
5. Temperature (The temperature at which the analysis was conducted)

Test Result Line
The components of the Test Result Line reading from left to right are:

1. Test Mode
2. Record Number
3. Read Number (Each sample analyzed is assigned a Read number. For a B-Tox and Q-Tox test, the first sample analyzed is assigned a Read Number of 001. Each additional sample tested is assigned the next ascending Read number.)
4. Time (The time of the day the reading was taken.)
5. Results (Result for the test sample. The results of B-Tox and Q-Tox tests are reported as either "Light Loss" or "Light Gain" and the value shown is always a whole number expressed in percent (%). The ATP results are reported as "light units" and the value is always a whole number based on photon counts.

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ANALYZER MAINTENANCE

Maintenance of the DeltaTox Analyzer should be conducted on a monthly basis (assuming regular use of the analyzer) to insure that the analyzer performs optimally. The maintenance is simple, easy to perform and requires only a minimum amount of time (usually less than 10 minutes).

- **Cleaning the case exterior and keypad:**
  Use a sponge wetted with dilute commercial spray cleanser (i.e. Window, 409, etc.) and gently wipe the case surface and keypad. DO NOT use abrasive cleansers or pads.

- **Cleaning the Read Chamber and Read Chamber Window:**
  Open the Read Chamber lid. Using a cotton tipped swab saturated with alcohol, wipe down the inner walls of the Read Chamber thoroughly. Use several dry swabs to complete the process. Leave the Chamber lid open and allow several minutes for the final traces of alcohol to evaporate.

- **Storing the DeltaTox Analyzer for prolonged periods of time:**
  Remove the batteries. The time and date will need to be reset the next time the instrument is used. Data stored in the analyzer will remain in memory for several years, unless it is deleted.

- **Removing a broken cuvette from the Read Chamber:**
  The PMT and all associated electronics are separated from the Read Chamber so there is very little danger of device damage. Simply remove all glass fragments with tweezers and alcohol wetted cotton swabs.

  If the broken cuvette contained a particularly concentrated amount of sample, it might be necessary to clean the Read Chamber several times with alcohol wetted cotton swabs.

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TROUBLESHOOTING

The effectiveness of troubleshooting is determined largely by an operator's concentration and patience in applying good common sense. Familiarity with the Operations Manual, and knowledge of the instrument operation and the characteristic responses when the instrument is operating normally, will provide greater success in troubleshooting. The following serves as an aid to troubleshooting for the various failure conditions that can be displayed. The possible causes for each failure condition are listed along with the corrective steps (listed in order of most to least likely resolution).

Error Message	**Possible Cause**	**Solution**
Screen displays "Dark current limit exceeded"	PMT Dark Current exceeds the manufacturer set maximum Dark Current operation level	1. Make sure a cuvette is not in a sample chamber 2. Cycle instrument power off/on 3. If these steps fail, contact the manufacturer
Screen displays "Close lid and latch"	The sample chamber shutter must be closed to continue operation	1. Close the latch and press the START key again 2. If instrument operation does not resume, insure the shutter latch is closed and cycle the power off/on 3. If these steps fail, contact the manufacturer
Screen displays "Switch or motor failure"	Problem with motor which moves the optical density filter or the 3 opto-reflective type switches to determine motor and shutter positioning	1. Replace instrument batteries or use the AC Power Adapter 2. If this step fails, contact the manufacturer
Screen displays "Unable to set PMT level"	Problem related to a function of the PMT Dark Current test	1. Close the latch and press the START key again 2. If this step fails, contact the manufacturer

Screen displays “PMT Over Range Condition”	Sample surpasses the dynamic range (greater than 120,000,000 photon counts)	1. If performing a TOX test rehydrate a new bottle of reagent 2. If performing an ATP test, dilute the sample 3. If these steps fail, contact the manufacturer
Screen displays “Battery Level Low”	Batter power insufficient	1. Replace the batteries 2. Use the AC Power Adapter 3. If these steps fail, contact the manufacturer
Screen displays “Manufacturing Test in progress…fail”	Instrument has failed one or more of the series of automatic diagnostic start up test used to assure that all systems are working properly	1. Make sure that a cuvette was not left in the sample chamber from a previous test 2. Make sure the latch is closed, then cycle the power off/on 3 If these steps fail, contact the manufacturer
Screen displays “Failed Calibration test 1” or “Failed Calibration test 2” or “Failed Calibration test 3”	Problem with the neutral density filter which is moved in from of the PMT under proper operational conditions. Calibration tests #1, #2, and #3 are performed to insure the integrity of the filter and optics	1. Make sure a cuvette is not in the sample chamber 2. If problem persists, then clean the sample chamber window 3. If these steps fail, contact the manufacturer
Screen displays “Memory too Full…download records	Memory is full and all data and must be downloaded	1. Download the data and then delete records from the Analyzer 2. If these steps fail, contact the manufacturer
Screen displays “Temperature High by xx.x degrees”	Instrument body temperature exceeds 40°C	1. Place the instrument in an environment with an ambient temperature between 10°C and 28°C and wait for the instrument to adjust to the new temperature before starting another test 2. If this step fails, contact the manufacturer

Screen displays “Temperature Low by xx.x degrees”	Instrument body below 5°C	1. Place the instrument in an environment with an ambient temperature between 10°C and 28°C and wait for the instrument to adjust to the new temperature before starting another test 2. If this step fails, contact the manufacturer

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SERVICE ASSISTANCE

Strategic Diagnostics, Inc. Technical Support Services	800-544-8881
Strategic Diagnostics, Inc. Customer Service and Ordering	800-544-8881

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SPECIFICATIONS

Size:	10” x 6” x 4.5” (25.4cm x 15.2cm x 11.4cm)
Weight:	5.0 lbs. (2.3 kg); 6.0 lbs. (2.7 kg) with batteries
Power:	Either 4 standard “C” cell batteries or a universal Power adapter (5.0 Vdc @ 4 amps)
Operational Temperature:	Ambient (10°C - 28°C)
Dynamic Test Range:	1 to 120 million counts (approx.)
Approvals:	CE (European Community)
Display Output:	LCD – 2 columns of 20 characters each
Data I/O:	9 pin female serial output port for the download of data and the upload of parameter settings
Filter Count Threshold:	5000 counts in 0.1 second (50 kilo counts / second)
Data Storage:	6.5k byte storage area (approx. 600 reads)
Data Handling:	Stand alone or download capability to PC; built in software prompts operational steps, records light measurements and automatically calculates results for immediate review and further analysis
Testing Concentrations:	90% or any lower concentration
Test Reagent:	Freeze-dried luminescent bacteria (*Vibrio fischeri*)
Reagent Storage:	Freeze-dried -15°C to -25°C Rehydrated 2 hours (ambient temperature) Cycled From -20°C to 20°C (a maximum of 3 cycles)
Test Modes:	Toxicity (Q-Tox and B-Tox) and ATP measurement
Test Durations:	5-20 minute exposure
Test Measurement Criterion:	Light output by test reagent measured after timed exposure to a sample
Results Display:	Percent light loss or gain for toxicity test, or light unit (photon) count (for ATP measurements)

Repeatability (Precision):	<20% coefficient of variation for B-Tox and Q-Tox mode testing
Sensitivity and Range:	The analyzer can detect photon counts from the low 10’s up to 120 million

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APPENDIX

Results Computation for Q-Tox and B-Tox Mode Testing

Q-Tox Procedure
The formula for calculating % Light Loss or % Light Gain is as follows:

$\%\ \text{Light Loss} = [(C_t - S_t) / C_t] \times 100$ (the calculated value is positive and reported as a positive %)

$\%\ \text{Light Loss} = [(C_t - S_t) / C_t] \times 100$ (the calculated value is negative and reported as a positive %)

Where C_t = light reading (photon count) for control at time "t" where t could be 5, 10, 15 or 20 minutes.
S_t = light reading (photon count) for sample at time "t" where t could be 5,10,15 or 20 minutes.

Example: Control = $C_{5\ min}$= 27,245,833
Sample = $S_{5\ min}$= 15,829,667

$\%\ \text{Light Loss} = [(27{,}245{,}833 - 15{,}829{,}667) / 27{,}245{,}833] \times 100$
= 42 (rounded to whole value)

B-Tox Procedure
The formula for calculating % Light Loss or % Light Gain is as follows:

$\%\ \text{Light Loss} = \{1 - [(S_t \times C_o) / (S_o \times C_t)]\} \times 100$ (the calculated value is positive and reported as a positive %)

$\%\ \text{Light Gain} = \{1 - [(S_t \times C_o) / (S_o \times C_t)]\} \times 100$ (the calculated value is negative and reported as a positive %)

Where C_o = light reading (photon count) for control at time zero
C_t = light reading (photon count) for control at time "t" where t could be 5, 10, 15 or 20 minutes
S_o = light reading (photon count) for sample at time zero
S_t = light reading (photon count) for sample at time "t" where t could be 5, 10, 15 or 20 minutes

Example: Control = $C_{0\ min}$ = 19,411,167 Sample = $S_{0\ min}$ = 19,285,500
Control = $C_{15\ min}$ = 15,149,333 Sample = $S_{15\ min}$ = 11,847,333

$\%\ \text{Light Loss} = \{1 - [(11{,}847{,}333 \times 19{,}411{,}167) / (19{,}285{,}500 \times 15{,}149{,}333)]\} \times 100$
= 21 (rounded to whole value)

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Q-Tox and B-Tox Data Interpretation and Precision

- The DeltaTox test is a single point method with no temperature control, therefore some data variation may be observed (depending on environmental conditions) as part of normal operation
- Depending on the type of sample and level of toxicity, the testing of environmental or chemical samples with the DeltaTox system, Q-Tox and B-Tox procedures will provide results either as % Light Loss or % Light Gain
- The amount of % light loss (an indication of metabolic inhibition in the test organisms) indicates the degree of sample toxicity. For example, a 40% light loss and a 75% light loss indicates a moderate to high level of sample toxicity
- The amount of % light gain (an indication of enhanced activity in the test organisms) indicates the degree of light stimulation and bioreactivity. For example, a 18% light gain and 52% light gain indicate a low to high level of stimulation from the sample.
- In general, samples with low levels of toxicity may produce low % light gain (stimulation) results
- For practical purposes and the interpretation of DeltaTox test results, the following terms can be considered synonymous:

  % Light Loss = % Inhibition
  % Light Gain = % Stimulation

- The B-Tox and Q-Tox procedures will give nearly equivalent results, but the B-Tox procedure will generally be the more accurate procedure
- The B-Tox and Q-Tox procedures typically yield a coefficient of variation <20%, but the B-Tox procedure, in general, will provide greater precision in test results
- For specific data uses and applications, it is recommended to develop "Baseline Data" for establishing relevant "Toxicity Limits"
- <u>Light Gain / Stimulation</u>:

  - Light stimulation will normally be caused by hormesis and sometimes by nutrients and salts present in test samples
  - Light stimulation indicates the degree of bioreactivity, biological response or stress conditions of test organisms to contaminants present in samples
  - The % light gain (stimulation) results may also indicate increased sensitivity of the DeltaTox test for screening environmental samples with low toxicity levels
  - Hormesis is a term that describes the increased activity in organisms when they are exposed to low levels of toxicants. Hormesis can also be described as an event where the test organism is stimulated at the beginning and then the event later becomes toxic to the organism (due to different metabolic reactions/pathways). Hormesis is well documented in the toxicity literature.

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Preparation of Sodium Thiosulfate Stock Solution (1% w/v)

1. Weigh out 1.0 g of sodium thiosulfate ($Na_2S_2O_3$) and add to a clean 100 mL volumetric flask
2. Add 60 mL of distilled or deionized water to the flask. Swirl the flask to dissolve the sodium thiosulfate
3. Bring the volume up to the 100 mL mark of the flask with distilled or deionized water. Cover the opening of the flask with Parafilm and invert the flask several times for complete mixing
4. Keep the stock solution stored in the refrigerator between 2°C to 8°C. The solution will remain stable for up to 2 months.

Dechlorination with Sodium Thiosulfate

1. To dechlorinate a sample, add 1 part of 1% sodium thiosulfate solution to 100 parts of sample. (For example, add 100 µL of 1% sodium thiosulfate solution to 10 mL of sample.)
2. Mix the sample.
3. Test the sample immediately for toxicity testing.

Adjusting pH of Sample

1. Measure the pH of the sample.
2. If the pH of the sample is below 6.0 or above 8.0, then adjust the pH of the sample to within the range of 6.0 to 8.0 for toxicity testing as per specific/regulatory requirements.
3. If the sample pH is below 6.0, adjust the pH to 6.0 using a NaOH solution. For course adjustment, use a 5 N concentration, for fine adjustment, use a 0.5 n concentration. If over-titration occurs, discard the sample and start again.
4. If the sample pH is above 8.0, adjust the pH to 8.0 using a HCl solution. For course adjustment, use a 5 N concentration; for fine adjustment, use a 0.5 N concentration. If over-titration occurs, discard the sample and start again.

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