2886888

Eclox™ Rapid Response Test Kit

User Manual

01/2018, Edition 5

Table of Contents

Section 1 Specifications 7

Section 2 General information 9
2.1 Safety information 9
2.1.1 Use of hazard information 9
2.2 Product overview 10
2.2.1 Test descriptions 10
2.2.2 Test setup 12
2.3 Unpack the instrument 13

Section 3 Luminometer 15
3.1 Overview 15
3.2 Prepare the luminometer for use 16
3.2.1 Test the operation 16
3.2.2 Erase the results saved on the luminometer 17
3.2.3 Set the measurement range 18
3.3 Change the default settings 20
3.3.1 Set the LCD contrast 20
3.3.2 Set the waiting time and measuring time 20
3.4 Connect the luminometer to a printer 21
3.5 Connect the luminometer to a computer 23
3.6 Install LUMISsoft on the computer 23

Section 4 Chemiluminescence Toxicity Test 25
4.1 Overview 25
4.2 Prepare the reagents for luminometer calibration and sample testing 25
4.2.1 Prepare CT Reagent 2 26
4.2.2 Prepare CT Reagent 3 26
4.3 Calibrate the luminometer 28
4.4 Measure pollutants in the water sample 30
4.5 Show the previous results 33
4.6 Send previous results to a computer 33

Section 5 EZ Arsenic Test Kit 35
5.1 Test preparation 35
5.2 EZ Arsenic, 0–500 ppb (0, 10, 25, 50, 100, 250, 500) 35
5.3 EZ Arsenic, 0–4000 ppb (0, 35, 75, 175, 1500, 4000) 37
5.4 Interferences 37
5.4.1 Optional procedure for removing sulfide 38

Table of Contents

Section 6 Pesticide/Nerve Agent Test 39
6.1 Pesticide/Nerve Agent procedure 39

Section 7 Free and Total Chlorine Test 41
7.1 Test preparation 41
7.2 Free or total chlorine procedure, 0–3.4 mg/L Cl2 41

Section 8 Color Test 43
8.1 Test preparation 43
8.2 Color, apparent (0–100 APHA platinum cobalt color units) 43
8.3 Color, apparent (0–500 APHA platinum cobalt color units) 45

Section 9 Pocket Pro™+ Multi 2 Tester 47
9.1 pH or TDS measurement 47
9.2 pH calibration 49
9.3 TDS/Conductivity calibration 50

Section 10 Luminescent Bacteria Toxicity Test: Screening/LIMIT measure 53
10.1 Overview 53
10.2 Accuracy 54
10.3 Reagent description 54
10.3.1 Quality assurance test 54
10.4 Reagent storage and preservation 55
10.5 Prepare the reagent 55
10.6 Prepare the stock suspension using the LCK491 reagent 55
10.7 Prepare the test suspension 58
10.8 Sample collection, storage and preservation 59
10.9 Interferences 59
10.10 Prepare the sample 60
10.11 Prepare the test tubes 61
10.12 Measure the toxicity of the sample dilutions 64
10.13 Show or send previous results 69
10.13.1 Description of screening luminescence results 69
10.13.2 Description of LIMIT measure results 70
10.13.3 Show or send screening luminescence results 70
10.13.4 Show or send LIMIT measure results 71

Section 11 Luminescent Bacteria Toxicity Test ISO 11348 part 3 73
11.1 Overview 73
11.2 Accuracy 74
11.3 Thermostat and PC software requirements 74

11.4 Reagent description 74
11.5 Reagent storage and preservation 75
11.6 Prepare the reagent 75
11.6.1 Prepare the stock suspension using the LCK491 reagent 75
11.6.2 Prepare the test suspension 77
11.7 Sample collection, storage and preservation 83
11.8 Interferences 83
11.9 Prepare the sample 84
11.10 Prepare the dilutions series 85
11.10.1 Prepare a 9 dilution series (D 2 values and higher) 85
11.10.2 Prepare a 3 dilution series (D 2 values and higher) 89
11.10.3 Prepare a 9 dilution series (D 1 values and higher) 93
11.10.4 Prepare a 3 dilutions series (D 1 values and higher) 98
11.11 Measure the light intensity of the test suspension 101
11.12 Measure the light intensity of the test suspension after the sample dilutions are added 106
11.13 Show or send previous results 108

Section 12 Maintenance 111
12.1 General maintenance 111
12.1.1 Cleaning the kit 111
12.1.2 Cleaning the luminometer 111
12.2 Decontamination 111
12.3 Battery replacement 112
12.3.1 Luminometer battery replacement 112

Section 13 Troubleshooting 115

Section 14 Replacement parts and accessories 117
Luminescent bacteria risks 121
Risk specifications 121
Biosafety Level 1 information 121
Disposal 122

Section 1 Specifications

Specifications are subject to change without notice.

General	
Dimensions	520 x 450 x 215 mm (20.5 x 17.5 x 8.5 in.)
Weight	9 kg (20 lb), fully loaded
Temperature	Tested from –20 to 55 °C (–4 to 131 °F)
Modes	Chemiluminescence Toxicity Testing Luminescent Bacteria Toxicity Testing
Chemical Tests	Arsenic, Chemiluminescence Toxicity, Chlorine, Color, Nerve Agents, Pesticides, pH, Total Dissolved Solids (Conductivity)
Certification	CE marked
Luminometer—Water and chemical warfare agent resistant	
Weight	1.4 kg (3.09 lb), including batteries
Dimensions	230 x 77 x 125 mm (9.1 x 3 x 4.92 in.)
Temperature	Tested from –20 to 55 °C
Battery Type	4 AA alkaline, at least 250 test per set of batteries
Display	Graphical LCD display with backlight for low light conditions
Data Logging	Up to 60 test results recorded in full detail Up to 100 luminescent measurements for Luminescent Bacteria Toxicity Test Up to 100 screening results for Luminescent Bacteria Toxicity Test
Download Capability	RS232
Power	Battery powered alkaline cell, lithium cell, AA CAUTION: For quality and safety reasons, only alkaline batteries should be used with this instrument. Use of other batteries may reduce the functioning of and/or damage the instrument electronics by overloading the electronics, or, depending on the battery type, can cause fire or an explosion.
Warranty	One year
Light detection for the Luminescent Bacteria Toxicity Test	Two decades in two different ranges: 20 to 1000 relative units (default mode) 20 to 2000 relative units Precision: 2% coefficient of variation
Arsenic	
Range	0 to 4 mg/L
Limit of detection	0.01 mg/L

Chlorine (free)	
Range	0 to 3.4 mg/L
Chlorine (total)	
Range	0 to 3.4 mg/L
Color	
Range	0 to 100, 0 to 500 APHA Platinum-Cobalt Color units
Pocket Pro™+ Multi 2 Tester	
Range	**pH**: 0.0 to 14.0 pH **TDS**: Auto-ranging (0.0 to 99.9 ppm, 100 to 999 ppm, 1.00 to 10.00 ppt)
Accuracy	**TDS**: ± 1%
Operating temperature	0 to 50 °C
Battery life	250 hours with backlight continuously on
Enclosure	IP67 Rated, waterproof (immersible), dust proof
Warranty	1 year for the tester and 6 months for replacement sensor for manufacturing faults only. Damage from use is not covered.

Section 2 General information

In no event will the manufacturer be liable for direct, indirect, special, incidental or consequential damages resulting from any defect or omission in this manual. The manufacturer reserves the right to make changes in this manual and the products it describes at any time, without notice or obligation. Revised editions are found on the manufacturer's website.

2.1 Safety information

Important Note: *The manufacturer is not responsible for any damages due to misapplication or misuse of this product including, without limitation, direct, incidental and consequential damages, and disclaims such damages to the full extent permitted under applicable law. The user is solely responsible to identify critical application risks and install appropriate mechanisms to protect processes during a possible equipment malfunction.*

Please read this entire manual before unpacking, setting up, or operating this equipment. Pay attention to all danger and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment.

Make sure that the protection provided by this equipment is not impaired. Do not use or install this equipment in any manner other than that specified in this manual.

CAUTION: Chemical exposure hazard. Obey laboratory safety procedures and wear all of the personal protective equipment appropriate to the chemicals that are handled. Refer to the current safety data sheets (MSDS/SDS) for safety protocols.

2.1.1 Use of hazard information

DANGER
Indicates a potentially or imminently hazardous situation which, if not avoided, will result in death or serious injury.

WARNING
Indicates a potentially or imminently hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION
Indicates a potentially hazardous situation that may result in minor or moderate injury.

Important Note: *Indicates a situation which, if not avoided, may cause damage to the instrument. Information that requires special emphasis.*

2.1.2 Precautionary labels

Read all labels and tags attached to the instrument. Personal injury or damage to the instrument could occur if not observed. A symbol on the instrument is referenced in the manual with a precautionary statement.

	This is the safety alert symbol. Obey all safety messages that follow this symbol to avoid potential injury. If on the instrument, refer to the instruction manual for operation or safety information.
	Electrical equipment marked with this symbol may not be disposed of in European domestic or public disposal systems. Return old or end-of-life equipment to the manufacturer for disposal at no charge to the user.
	This symbol indicates that a risk of electrical shock and/or electrocution exists.
	This symbol indicates the need for protective eye wear.

2.2 Product overview

The Eclox™ Rapid Response Test Kit is used to do first line water testing. The kit is a generic qualitative test that gives a broad indication of water quality. To do a proper toxicity test, identify a baseline using the product in the waters to be tested.

The kit can be used to:

- Compare and prioritize possible source waters that might be used in a purification process to make drinking water.
- Give information to an operator to help the operator identify the correct water treatment process for the quality of the source water available.
- Be a regular quality assurance test on the drinking water made or the water given for drinking and, if applicable, on the source water.

Other configurations are available, including the Eclox ‘toxicity only’ kit and the luminometer by itself.

2.2.1 Test descriptions

Eight tests are included in the Eclox™ Rapid Response Test Kit:

- **Chemiluminescence Toxicity Test—**shows the toxicity of the water sample. This test uses a plant enzyme, which creates light (chemiluminescence) when mixed with other reagents. Pollutants in the water sample prevent this reaction, which

reduces the amount of light that is created. The more pollutant that is in the sample, the less light that is created. The light made by the sample water is compared to a pure water reference and the percentage inhibition of the light made is measured and made known.

- **Arsenic Test—**measures the arsenic content of the water sample. Arsenic is a common poison and industrial pollutant. Arsenic is also in chemical warfare (CW) agents, such as Lewisite. The result of this test can be read in mg/L from a comparison chart.

- **Pesticide/Nerve Agent Test**—gives a YES/NO answer if pesticide/nerve agents are in the water sample.

- **Chlorine Test**—measures how much free chlorine is in the water sample and gives the results in mg/L. Systems using monochloramines may choose to monitor total chlorine.

  Chlorine is frequently used to disinfect water for human consumption. The quantity of chlorine used must be carefully controlled and the free residual concentration of the chlorine in the water gives a useful means of monitoring the effectiveness of the water treatment done. Chlorinated water can, however, cause damage to the Reverse Osmosis (RO) filter of water purification equipment and should not be used as a source water in a RO type of process.

- **Color Test**—a comparison test that compares the water sample with a calibrated gradient color disc. The results are read in platinum cobalt (Pt-Co) color units. Color in water may be caused by the presence of natural metallic ions (iron and manganese), peat materials, plankton, weeds and industrial wastes.

- **Total Dissolved Solids (TDS) Test**—measures the level of dissolved solids in the water sample. TDS and the conductivity of a sample are related. The TDS is approximately 0.7 of the conductivity result ($\mu S/cm^3$).

- **pH Test**—measures the pH level of the water sample.

- **Luminescent Bacteria Toxicity Test (optional)—**a biotest that measures the toxicity of environmental samples. Toxicity is a biological or biochemical sum parameter that cannot be measured by chemical analysis. Toxicity is a measure of the effect of a sample on living organisms, biological systems and enzymes. Other biotests such as fish, daphnia and algae tests are more complex and, because other biotests use higher living organisms, are also controversial. In the practice of environmental analysis, the Luminescent Bacteria Toxicity Test has shown to be fast, simple, reliable and sensitive.

  The Luminescent Bacteria Toxicity Test uses natural bacteria that make light. Toxic samples decrease the amount of light the bacteria make. The more toxic the sample, the less light the bacteria make. The amount of light that is made by the

bacteria after exposure to a sample is compared to the amount of light made by the bacteria after exposure to a control to identify the percent inhibition value of the sample. The control contains no sample but a non-toxic reagent blank (2% NaCl solution).

The reagent set is sold independently.

The Luminescent Bacteria Toxicity (LBT) Test can be done using either the:

- **Measurement Luminescence procedure**—used in the lab when a thorough assessment of the inhibitory effects of a sample is necessary. Use the LBT measurement luminescence procedure if the test needs to be done according to ISO 11348.
- **Screening Luminescence procedure**—used in the field or in an emergency situation when a rapid assessment of the inhibitory effects of a sample is necessary. The LBT screening luminescence procedure is a simplified test procedure that uses the same reagents according to ISO 11348 but at ambient conditions.
- **LIMIT measure procedure**—the same as the screening luminescence procedure. However, the LIMIT measure procedure lets the user set a LIMIT value on the luminometer. The LIMIT value is used by the luminometer to include in the test results whether the percent inhibition is above or below the LIMIT value.

2.2.2 Test setup

There are three basic operations to be done when using the kit:

- **Pre-deployment checks**—complete before starting on a series of tests. Refer to the Quick Start Guide (28878-88) on the lid of the case.
- **Luminometer test**—test the operation of the luminometer before Chemiluminescence Toxicity Tests or Luminescent Bacteria Toxicity Tests are done.
- **Luminometer calibration**—calibrate the luminometer each day before Chemiluminescence Toxicity Tests are done.
- **Measure samples**—measure the samples with the tests.

2.3 Unpack the instrument

Remove the Eclox Rapid Response Test Kit from the shipping carton and check it for any visible damage. If any items are missing or damaged, contact the manufacturer or a sales representative immediately.

- Carrying case
- Blue pipette, 1000 µL
- Yellow pipette, 100 µL
- Pocket Pro™+ Multi 2 Tester
- Color comparator box
- Color viewing tubes, plastic (2x)
- Longpath viewing adapter
- Color discs (2x)
- EZ Arsenic Reagent 1 Powder Pillows
- EZ Arsenic Reagent 2 Powder Pillows
- EZ Arsenic Test Strips
- Arsenic reaction bottle with cap
- DPD Free Chlorine Powder Pillows
- DPD Total Chlorine Powder Pillows
- Sample cell, plastic
- Sodium chloride, 85.47 mg/L (100 mL)
- pH 4.01 SINGLET™
- pH 7 SINGLET™
- Waste bag with zipper
- Waste bottle, 250 mL
- Beaker, 50 mL
- Pesticide/Nerve Agent Test Strips
- Pesticide test clip
- Luminometer
- Serial cable for luminometer
- Batteries, AA, Alkaline
- Test record pad
- Cuvette and pipette tip set, blue
- Cuvette and pipette tip set, yellow
- Cuvette holder
- LUMISsoft installation CD
- Chemiluminescence Reagent Set

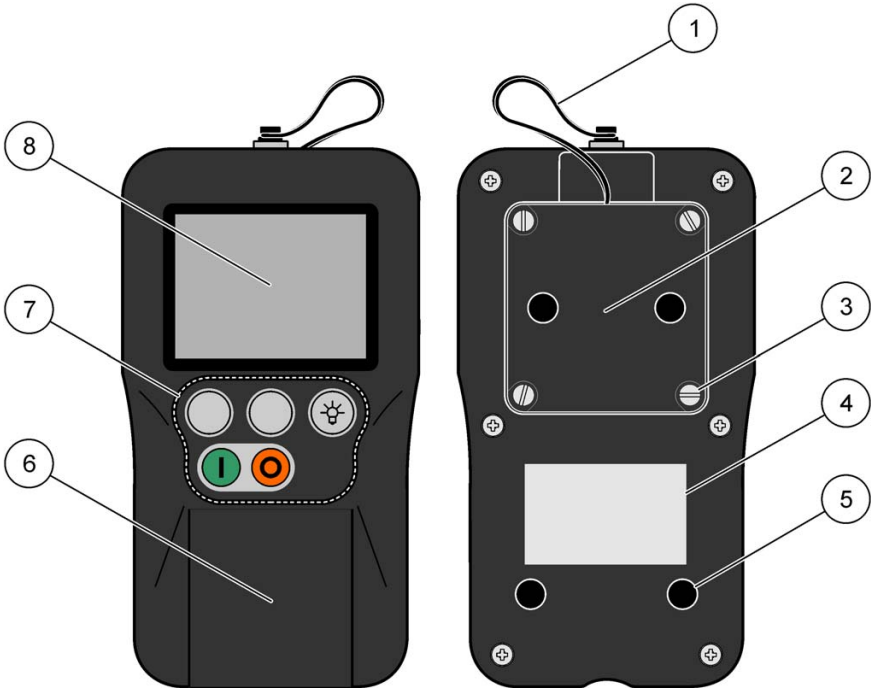
Section 3 Luminometer

3.1 Overview

The Eclox luminometer is used with the Chemiluminescence Toxicity Test and the Luminescent Bacteria Toxicity Test to measure and record relative light units made by the reagents when exposed to samples.

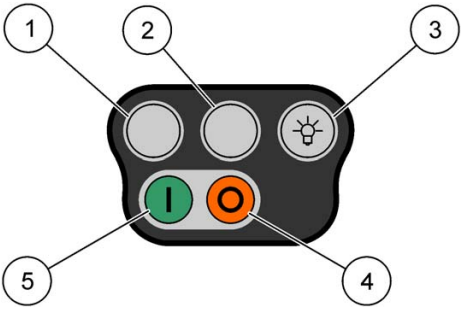
The Eclox luminometer is made for use under extreme field conditions. The Eclox luminometer components are rugged, easy to use and reliable (refer to Figure 1).

Figure 1 Luminometer components



1.	Lanyard	**5.**	Non-slip feet
2.	Battery compartment	**6.**	Cell lid
3.	Battery compartment screws	**7.**	Function keys (Figure 2)
4.	Label	**8.**	Display

Figure 2 Function keys



Item number	Description	Function
1	Soft key	Does the action on the display directly above the key.
2	Soft key	Does the action on the display directly above the key.
3	Back light button	Illuminates the display.
4	Off button	Removes power to the instrument.
5	On button	Applies power to the instrument.

3.2 Prepare the luminometer for use

Do the procedures in this section before each deployment to prepare the luminometer for use.

3.2.1 Test the operation

Do this procedure to make sure that the luminometer is operating correctly. If the luminometer passes all the tests done in this procedure, it is operating correctly.

To test the operation of the luminometer:

1. Open the hinged cell lid of the luminometer and make sure that a sample is not in the cell.
2. Remove the black test tube holder from the cell. Make sure that the cell is clean and free from debris.
3. Put the black test tube holder into the cell and close the cell lid.
4. Push **ON** (green button) for several seconds to apply power to the instrument. If the instrument does not energize, replace the batteries (refer to section 12.3 on page 112).

The instrument does built-in tests that make sure that the electronics and software are operating correctly.

5. Make sure that all tests show a PASS on the display. If an error is shown on the display, refer to Troubleshooting on page 115.

6. Push **PROCEED**.

   The Main Menu is shown.

7. Select either ECLOX or Luminescent Bacteria Test and push **ENTER**. Either option can be selected for this procedure.

8. Check the battery level symbol at the top right corner of the display. Make sure that at least two level bars are shown. If two or more bars are not shown, replace the batteries in the instrument and go back to step 4.

9. Select System Tests and push **ENTER**.

   The System Tests Menu is shown.

10. Push **ENTER** to select Check Signal Level.

    The Signal Level screen is shown.

11. Push **PROCEED** to do a cell zeroing test. When the instrument has passed the test, the Signal Level screen is shown again.

12. Push and hold **TEST**. Make sure that the signal level shown is above the minimum and below the maximum. If the signal level is not above the minimum, contact the manufacturer for technical support.

13. Push and hold the back light button. Make sure that the instrument display light comes on.

14. Push and hold **QUIT** for a few seconds.

    The Systems Test Menu screen is shown.

15. Select Return to the ECLOX Main Menu (or LBT Main Menu) and push **ENTER**.

3.2.2 Erase the results saved on the luminometer

1. Push **ON** (green button) for several seconds to apply power to the instrument.

   The instrument does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED**.

   The Main Menu is shown.

3. To erase all the Chemiluminescence Toxicity Test measurements, select ECLOX and push **ENTER**.

4. To erase all the Luminescent Bacteria Test measurements, select Luminescent Bacteria Test and push **ENTER**.

5. Select Set-Up and push **ENTER**.

   The Set-Up Menu is shown.

6. Select Clear All Measurements and push **ENTER**. Push **YES** to confirm.

   All saved measurements on the luminometer are erased.

7. Push **PROCEED**.

   The Set-Up Menu is shown.

3.2.3 Set the measurement range

Set the luminometer measurement range to 0–1000 light units (normal use) or 0–2000 light units (measurement of sea water samples).

If the measuring value is marked with an * (e.g., 1020*) or the lumiometer shows Detector Overload, the measurement is above the set measurement range. If this occurs, change the measurement range to 0–2000 light units and do the reading again.

Statistical research of each measurement range has shown that the standard deviation of the 0–2000 range is less than the standard deviaton of the 0–1000 range and that the precision at the 0–2000 range may be better. In comparison studies of each range, the phenol standardization check showed equal results to the expected 50% inhibition range.

Non-polluted sea water samples “enhance” the signal (give a higher light inhibition of approximately -40%). As the sea water becomes more polluted the percentage inhibition increases (towards 0%) and then goes positive (e.g., 10%). Sea water which is very polluted gives a signal similar to that of fresh water which is very polluted (e.g., 70–100% light inhibition).

3.2.3.1 Eclox chemiluminescence test

To show or change the measurement range for the Eclox chemiluminescence test:

1. Push **ON** (green button) to apply power to the instrument.

   The instrument does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED**.

   The Main Menu is shown.

3. Select ECLOX and push **ENTER**.

   The ECLOX Main Menu is shown.

4. Select Set-Up and push **ENTER**.

The Set-up Menu is shown.

5. Select Set Measurement Range and push **ENTER**.

   The current range is shown.

6. Push **YES** to confirm.

7. To change the range, push **CHANGE**

8. Push **STORE** to save the change.

   The Set-up Menu is shown.

3.2.3.2 Luminescent Bacteria Test

If the measurement range is set to the 0–2000 light units and the measuring value is marked with an * (e.g., 2010*) or the lumiometer shows Detector Overload, the measurement is above the set measurement range. If this occurs, dilute the bacterial stock suspension with Diluent and do the reading again.

To show or change the measurement range for the Luminescent Bacteria Test:

1. Push **ON** (green button) to apply power to the instrument.

   The instrument does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED**.

   The Main Menu is shown.

3. Select LUMINESCENT BACTERIA TEST and push **ENTER**.

   The LBT Main Menu is shown.

4. Select Set-Up and push **ENTER**.

   The LBT Set-Up Menu is shown.

5. Select Set Measurement Range and push **ENTER**.

   The current range is shown.

6. Push **YES** to confirm.

7. To change the range, push **CHANGE**

8. Push **STORE** to save the change.

   The Set-up Menu is shown.

3.3 Change the default settings

3.3.1 Set the LCD contrast

The luminometer comes from the factory with the LCD contrast set correctly. Do this procedure to increase the luminometer LCD contrast for low light conditions.

1. Push **ON** for several seconds to apply power to the instrument.

   The instrument does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED.**

   The Main Menu is shown.

3. Select ECLOX or Luminescent Bacteria Test and push **ENTER**. Either option can be selected for this procedure.

4. Select Set-Up and push **ENTER**.

   The Set-up Menu is shown.

5. Select Set Screen Contrast and push **ENTER**.

   The Set Contrast screen is shown.

6. Push **DOWN** or **UP** to change the contrast. The screen shows the contrast level with a Max/Min indicator bar.

7. Push **DOWN** and **UP** at the same time to save the changes.

   The Set-up Menu is shown.

3.3.2 Set the waiting time and measuring time

This procedure only applies to the Luminescent Bacteria Test.

The measurement of the light intensity of luminescent bacteria is divided up into two parts:

- **Waiting time**—the amount of time the luminometer waits (after the test tube is put in, the lid is closed and **MEASURE** is pushed) before measuring the light intensity from the test tube. The luminometer needs to wait a few seconds to compensate for the high light level of the open lid.

- **Measuring time**—the amount of time the sample is measured by the luminometer.

Note: *There is no need to change the default settings of 8 seconds waiting time or 7 seconds measuring time unless HACH or HACH-LANGE customer service asks the user to do so.*

To show or change the waiting time and measuring time:

1. Push **ON** (green button) to apply power to the instrument.

   The instrument does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED**.

   The Main Menu is shown.

3. Select Luminescent Bacteria Test and push **ENTER**.

4. Select Set-Up and push **ENTER**.

   The Set-up Menu is shown.

5. To show or change the waiting time:

   a. Select Set Waiting Time and push **ENTER**.

      The current settings are shown.

   b. To change the waiting time, push **CHANGE**.

   c. Push **STORE** to save the change.

      The Set-up Menu is shown.

6. To show or change the measuring time:

   a. Select Set Measuring Time and push **ENTER**.

      The current settings are shown.

   b. To change the measuring time, push **CHANGE**.

   c. Push **STORE** to save the change.

      The Set-up Menu is shown.

3.4 Connect the luminometer to a printer

Luminometer measurements can be sent to a printer either during the test or after the test is done.

To connect the luminometer to a printer:

1. Pull out the plug that is attached to the lanyard.

2. If using a DPU-414 thermal printer, turn off the printer.

3. Connect the RS232 serial interface cable to the luminometer.

4. Put an adapter (DB9, 3 wires, male-male, 2-3, 3-2, 5-5. Cross over, not straight) on the other end of the RS232 serial interface cable.

5. Connect the RS232 serial interface cable to the printer.

6. If using a DPU-414 thermal printer:

   a. Configure the printer (refer to the printer manual for more information):

DIP switch	Switch	Position	Setting
DIP SW1	1	Off	Input = Serial
	2	On	Printing speed = High
	3	On	Auto loading = On
	4	Off	Auto LF = Off
	5	On	Setting command = Enable
	6	Off	Printing
	7	On	Density
	8	On	= 100%
DIP SW2	1	On	Printing columns = 40
	2	On	User font backup = On
	3	On	Character select = Normal
	4	On	Zero = Normal
	5	On	International
	6	On	Character
	7	Off	Set
	8	Off	= England
DIP SW3	1	On	Data length = 8 bits
	2	On	Parity settings = No
	3	On	Parity condition = Odd
	4	Off	Busy control = XON/XOFF
	5	Off	Baud
	6	On	Rate
	7	On	Select
	8	On	= 9600 bps

   b. When Continue? is shown, push **ON-LINE SW**.

c. When Write? is shown, push **PAPER FEED SW**.

d. Turn on the printer.

7. If not using a DPU-414 thermal printer, configure the printer:

Option	Setting
Data length	8 bits
Parity setting	No
Parity condition	Odd
Busy control	XON/XOFF
Baud rate	9600 bps

3.5 Connect the luminometer to a computer

To connect the luminometer to a computer:

1. Install the LUMISsoft software on the computer (refer to Install LUMISsoft on the computer on page 23).
2. Pull out the plug that is attached to the lanyard.
3. Connect the RS232 serial interface cable to the luminometer.
4. Connect the other end of the RS232 serial interface cable to the computer.

3.6 Install LUMISsoft on the computer

Install LUMISsoft on a computer by doing the instructions on the CD cover. A shortcut for LUMISsoft is added to the desktop during installation.

In the lab, LUMISsoft is used to automatically get LBT Measurement Luminescence procedure results from the luminometer during the test and put the values into LUMISsoft. LUMISsoft then does calculations according to ISO 11348.

LUMISsoft is also used to send previous results that are saved on the luminometer to a computer as a text file. The results can then be shown in graphical and tabular format using Microsoft Excel®. The user can also manually put test results shown on the luminometer into LUMISsoft to do calculations.

Section 4 Chemiluminescence Toxicity Test

The Chemiluminescence Toxicity Test uses the luminometer. Before doing the Chemiluminescence Toxicity Test, read section 3.1, Overview on page 15 and do the procedures in section 3.2, Prepare the luminometer for use on page 16.

4.1 Overview

The Chemiluminescence Toxicity Test and Luminescent Bacteria Toxicity Test both show the inhibitory effects of a sample. However, the Chemiluminescence Toxicity Test reagents are more rugged than the Luminescent Bacteria Toxicity Tests reagent and can be used under conditions where the Luminescent Bacteria Toxicity Tests reagent cannot be used.

The Chemiluminescence Toxicity Test reagents are stable for months even if stored under higher ambient temperatures up to 40 °C (Table 1). The Luminescent Bacteria Toxicity Tests reagent cannot be stored under those conditions.

4.2 Prepare the reagents for luminometer calibration and sample testing

Prepare the chemiluminescence test (CT) Reagents 2 and 3 at the beginning of deployment.

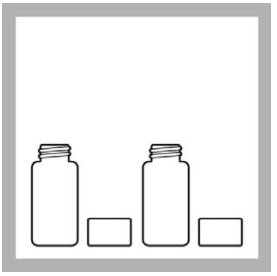
The chemiluminescence test (CT) Reagents 2 and 3 are temperature sensitive and degrade at high temperatures. For long-term storage, store the reagents in their stable forms. On the first day of testing, prepare the reagents for routine use.

Diluted reagents are stable for 72 hours. The life of the reagents is longer if the reagents are kept cool (e.g., in a refrigerator) and in a dark place. Before use, let the reagents get to ambient temperature.

Table 1 Chemiluminescence test reagent stability

Reagent	Refrigerated in a dark place	Raised Temperatures (+40 °C)
Reagent 1	12 to 18 months	1 year
Reagent 2 (stable form)	12 to 18 months	6 months
Reagent 2 (diluted form)	12 to 18 months	72 hours
Reagent 3 (stable form)	12 to 18 months	4 months
Reagent 3 (diluted form)	12 to 18 months	72 hours

4.2.1 Prepare CT Reagent 2

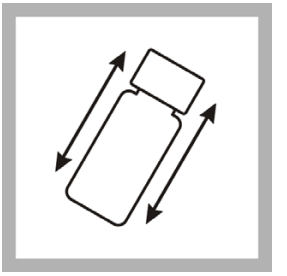


1. Remove the CT Reagent 2 buffer and CT Reagent 2 caps.

Note: *Do not open the bottles in heavy winds. The reagent in the CT Reagent 2 is small.*

Note: *Do not touch the reagent.*

2. Carefully put all of the CT Reagent 2 buffer into the CT Reagent 2 bottle.

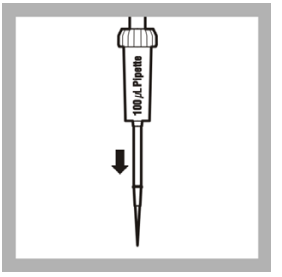


3. Put the caps back on the bottles. Shake the CT Reagent 2 bottle for 30 seconds. Let dissolve for 10 minutes before use.

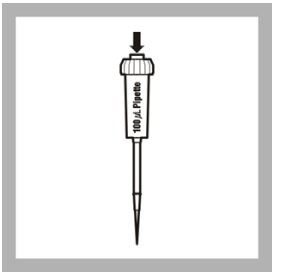
4.2.2 Prepare CT Reagent 3

1. Remove the CT Reagent 3 concentrate and CT Reagent 3 caps.

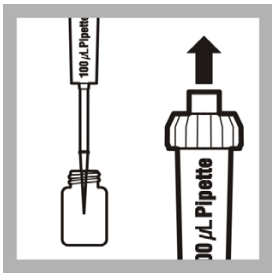
Note: *Make sure that the batch number for CT Reagent 3 is the same as the batch number used for CT Reagent 2.*



2. Push the end of the pipet into a clean 100 µL yellow pipet tip and remove from the box.

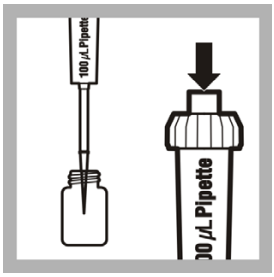


3. Push in the operating button on the top of the pipet to the stop.



4. Put the tip in the CT Reagent 3 concentrate 1 cm below the surface.

Slowly release the operating button to pull in the concentrate.

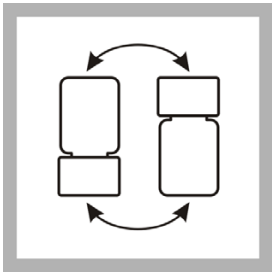


5. Put the tip into CT Reagent 3 and dispense the liquid by gently pushing in the operating button.

Put the tip into the liquid and then remove from the liquid.

6. Remove the tip from the pipet and put in the waste bag.

Put the pipet in the storage case.



7. Put the cap on CT Reagent 3.

Turn over the CT Reagent 3 bottle several times to mix the solution.

Note: *An ice chest can be used in the field to extend the life of the reagent.*

4.3 Calibrate the luminometer

Calibrate the luminometer before doing the Chemiluminescence Toxicity Test and after preparing the reagents.

The luminometer needs to be calibrated with every new batch of chemiluminescence reagents.

To calibrate the luminometer:

1. Push **ON** (green button) to apply power to the luminometer.

   The luminometer does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED**.

   The Main Menu is shown.

3. Select ECLOX and push **ENTER**.

   The ECLOX Main Menu is shown.

4. Push **ENTER** to select Measure.

   The Measure Menu is shown.

5. Select Measure Reference and push **ENTER**.

6. Open the luminometer lid and make sure a sample is not in the cell. Then close the lid.

7. Push **PROCEED**.

   The test status is shown. The test may go a few minutes before it is done.

8. When all the cell tests are done, push **PROCEED**.

9. Open the lid of the Cuvette and 1000 µL Pipette Tip Set.

10. Put one cuvette in the black cuvette holder.

11. Place a blue pipette tip on the blue pipette.

12. Completely push in the operating button on the pipette to the stop and put the pipette tip in deionized water about 1 cm below the surface.

13. Release the operating button slowly to pull in the deionized water into the pipette.

14. Touch the pipette tip against the side of the deionized water bottle to remove any drops from the outside of the tip.

15. Place the pipette tip into the cuvette and dispense the liquid into the cuvette by gently pushing in the operating button to the stop.

16. Put the pipette tip into the cuvette and remove the pipette from the cuvette to remove any drops from the outside of the tip.

17. Remove the lids from the CT Reagents 1, 2 and 3.

18. Put a yellow pipette tip on to the yellow pipette.

19. Put 100 µL of CT Reagent 1, 2 and 3 into the cuvette using the pipette. Use a new pipette tip for each reagent.

20. Open the lid of the luminometer.

21. Lift the cuvette from the holder and gently tap the cuvette two times to mix the solution.

22. Immediately put the cuvette into the luminometer cell and close the lid.

23. Push **PROCEED**.

    The luminometer automatically starts measuring. After four minutes, the screen count down timer displays DONE.

24. When the measurement is complete, remove the cuvette from the luminometer.

25. Put the solution in the cuvette into the waste bottle.

26. Put the cuvette into the waste bag.

27. If the reference is between 300 and 900, the calibration is complete.

28. If the reference is not between 300 and 900, push **PROCEED** and do the calibration procedure again.

Note: *New reagents may give a signal over 900. If the signal is 900 or over, change the measurement range to the 0–2000 range. Do not throw away the reagent set. If the signal is below 300, the reagents are probably unusable due to temperature sensitivity and new ones are required.*

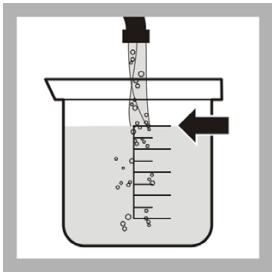
Note: *If the reagent baseline is reading over 1000 or the luminometer shows Detector Overload, change the measurement range to the 0–2000 range and continue. There is no need to throw away the chemiluminescent reagents.*

29. If the signal is below 300 again, add another 100 µL of CT Reagent 3 to the cuvette and do the calibration procedure again.

4.4 Measure pollutants in the water sample

Start with fresh reference everyday and with each new reagent set.

If the measured light units for the reagents are over 900, change the measurement range to the 0–2000 range and continue (refer to section 3.2.3, Set the measurement range on page 18).



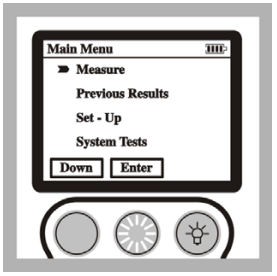
1. Fill the beaker with 50 mL of sample water.

2. If more than 0.4 mg/L chlorine is present, neutralize the sample by adding two drops of pre-conditioner reagent to the sample beaker.

Note: *Two drops of pre-conditioner reagent can neutralize up to 15 mg/L of chlorine.*

3. Push **ON** (green button) for several seconds to apply power to the luminometer.

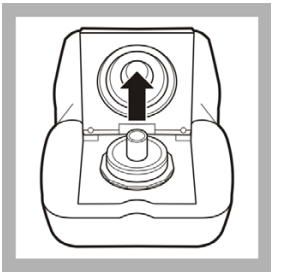
When the built-in tests are done, push **PROCEED**. The Main Menu is shown.



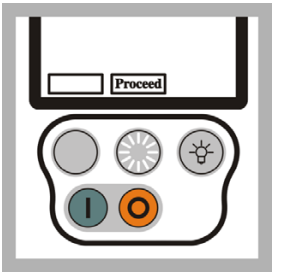
4. Select ECLOX and push **ENTER**.

Select Measure and push **ENTER**.

Select Measure Sample and push **ENTER**.

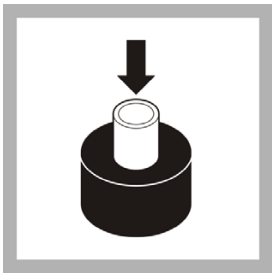


5. Open the luminometer lid and make sure that a sample is not in the cell. Close the lid.



6. Push **PROCEED** to show the test status.

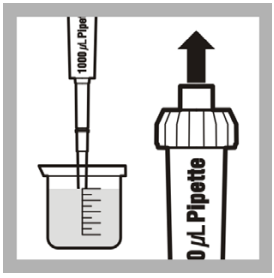
When the cell tests are done, push **PROCEED** again.



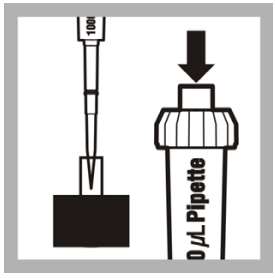
7. Put one cuvette from the Cuvettes and 1000 µL Pipet Tip Set into the black cuvette holder.

8. Put a blue pipette tip on the blue pipet.

9. Push in the operating button on the pipet to the stop.

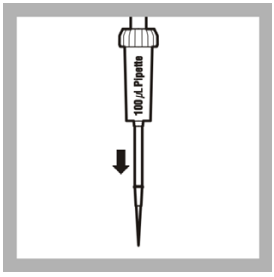


10. Put the tip in the sample water 1 cm below the surface. Slowly release the operating button to pull in the sample.



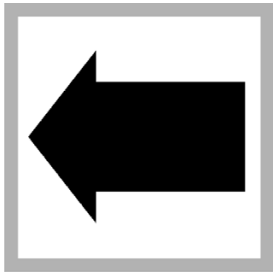
11. Put the tip into the cuvette and dispense the liquid by gently pushing in the operating button.

12. Remove the tip from the pipet and put in the waste bag. Put the pipet in the storage case.

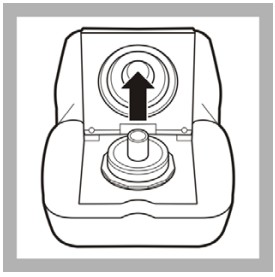


13. Put a yellow pipet tip on the yellow pipet.

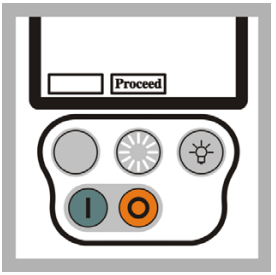
Use a new pipet tip for each reagent.



14. Do steps 9 to 12 to put 100 µL of CT Reagents 1, 2 and 3 into the cuvette.

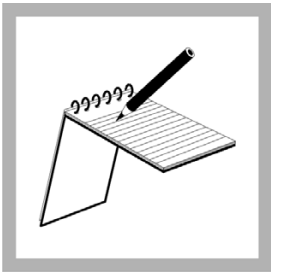


15. Open the luminometer lid. Remove the cuvette from the cuvette holder. Gently tap the cuvette twice to mix the solution. Put the cuvette in the luminometer cell.



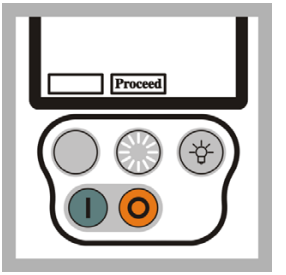
16. Close the lid. Push **PROCEED**.

The luminometer automatically starts measuring. After four minutes, the screen timer shows DONE.

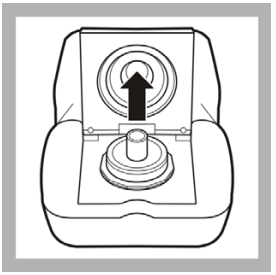


17. The Inhib% is shown on the screen. Record the Inhib% value and graph on the Test Record Sheet.

Note: *For sea water samples, the graph may appear higher than the reference and the Inhib% may be negative.*

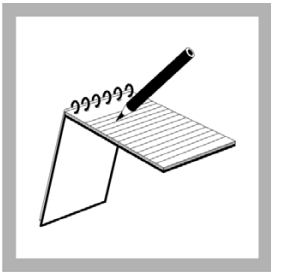


18. Push **PROCEED** to go back to the Measure Menu.



19. Remove the cuvette from the luminometer cell.

Put the solution into the waste bottle. Put the cuvette into the waste bag.



20. Sign the Test Record Sheet.

Put the sample from the beaker in the wastewater drain using local operating procedures.

4.5 Show the previous results

Up to 60 previous results (samples plus references) and graphs can be saved on the luminometer and then shown later on the luminometer.

To save previous results to a computer, refer to Send previous results to a computer on page 33.

To show previous results saved on the luminometer:

1. Push **ON** (green button) for several seconds to apply power to the luminometer.

   The luminometer does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED.**

   The Main Menu is shown.

3. Select ECLOX and push **ENTER**.

4. Select Previous Results and push **ENTER**.

   The Previous Results Menu is shown.

5. To show previous results:

   a. Select Recall Results and push **ENTER**.

   b. Push **MORE** to show more results.

   c. Push **QUIT** to go back to the Previous Results Menu.

6. To show previous graphs:

   a. Select Recall Graphs and push **ENTER**.

   b. Push **UP** to move through the saved samples.

   c. Push **SELECT** when the required graph number is shown to select a graph.

   d. Push **SELECT** again on the last selected graph number to select multiple graphs.

4.6 Send previous results to a computer

To send previous results to a computer:

1. Do the steps in Connect the luminometer to a computer on page 23.

2. Start LUMISsoft.

3. In LUMISsoft, select Transfer, Options, Interface Protocol, Connect.

4. Push **ON** (green button) for several seconds to apply power to the luminometer.

   The luminometer does built-in tests that make sure that the electronics and software are operating correctly.

5. When the built-in tests are done, push **PROCEED.**

   The Main Menu is shown.

6. Select ECLOX and push **ENTER**.

7. Select Previous Results and push **ENTER**.

   The Previous Results Menu is shown.

8. Select Download to PC and push **ENTER**.

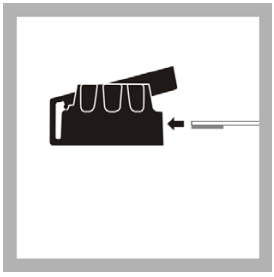
Section 5 EZ Arsenic Test Kit

DANGER: Hydrogen and arsine gasses are generated during the test. Work in a well-ventilated area away from open flames and other sources of ignition. Review Material Safety Data Sheets for safe handling, storage and disposal information.

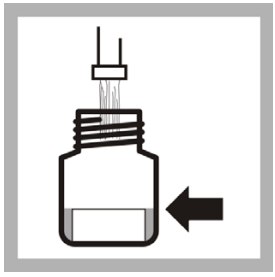
5.1 Test preparation

- For samples with sulfide greater than 15 ppb, follow Optional procedure for removing sulfide on page 38 before performing the test.
- Do not expose reacted strips to direct sunlight. Reaction products are photosensitive and may turn dark.
- Do not allow test strips to touch the reaction vessel solution. Test strips react with gases, not solution.
- Orient the test strip pad **paper side down** and **centered** over the hole in the black cap so the generated gases can make good contact with the pad.
- Two samples may be analyzed simultaneously with this kit.

5.2 EZ Arsenic, 0–500 ppb (0, 10, 25, 50, 100, 250, 500)



1. Insert a test strip into the cap so the pad completely covers the small opening. Close the flap and press to secure.

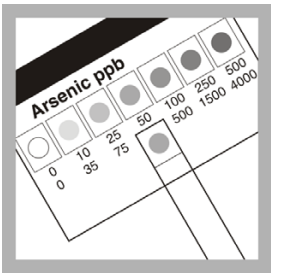


2. Fill the reaction bottle with sample to the fill line (50 mL).

3. Add one Reagent #1 and one Reagent #2 powder pillow to the sample.

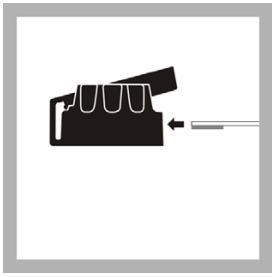
See Interferences on page 37.

4. Immediately attach the cap to the reaction bottle. Swirl continuously for 60 seconds.
Do not shake or invert or allow sample to get on the strip.
Wait 20 minutes. Swirl twice during the reaction period.



5. Remove the test strip and immediately compare the developed color to the chart on the test strip bottle (0–500 ppb row).
Read strips in the shade.

5.3 EZ Arsenic, 0–4000 ppb (0, 35, 75, 175, 1500, 4000)



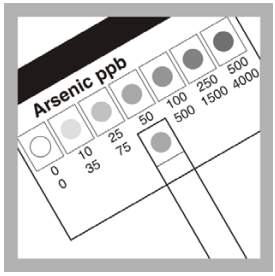
1. Insert a test strip into the cap so the pad completely covers the small opening. Close the flap and press to secure.

2. Fill the square measuring vial to the top with sample (9.6 mL). Pour the sample into the reaction bottle.

3. Add one Reagent #1 and one Reagent #2 powder pillow to the sample.

See Interferences on page 37.

4. Immediately attach the cap to the reaction bottle. Swirl continuously for 60 seconds.
Do not shake or invert or allow sample to get on the strip.
Wait 20 minutes.
Swirl twice during the reaction period.



5. Remove the test strip and immediately compare the developed color to the chart on the test strip bottle (0–4000 ppb row). Read strips in the shade.

5.4 Interferences

Table 2 Interfering substances

Ion or Substance	Concentration
Sulfide	>15 ppb[1]
Selenium	> 1 ppm

Table 2 Interfering substances

Ion or Substance	Concentration
Antimony	> 250 ppb
Tellurium	Likely to interfere, but not tested.
Acidity	< pH 5. Do not acid-preserve samples. If samples are below pH 5, adjust pH to between 5 and 6 before beginning test.
Nitric acid	Interferes with the reduction step. Do not use samples preserved with nitric acid because low results will be observed. If samples must be preserved, use HCl or sulfamic acid to adjust sample to pH 2. Adjust to pH 5–7 before running the test.

[1] See section 5.4.1 on page 38 for information on removing sulfide.

Table 3 Non-interfering substances

Ion or substance	Concentration
Hardness	1000 ppm as $CaCO_3$
Alkalinity	1000 ppm as $CaCO_3$
Iron	100 ppm
Temperature	10 to 40 °C (50 to 104 °F)

5.4.1 Optional procedure for removing sulfide

If a rotten egg smell is detected after adding reagent #1, sulfide is present at interfering levels. Complete the following steps to remove the sulfide before beginning the test procedure:

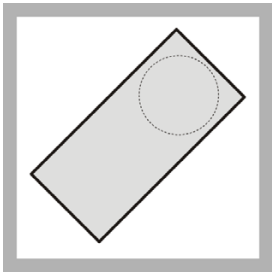
1. Tear off a small piece of cotton and form a ball the size of a pea.
2. Saturate the cotton with a few drops of lead acetate. Squeeze the excess liquid out of the cotton, leaving it damp.
3. Press the saturated cotton ball into the small opening of the reaction bottle cap from the bottom. Be sure that the cotton is firmly in place and that a gap remains between the cotton and the top surface of the cap.
4. Insert the test strip as detailed in step 1 of the 0–500 or 0–4000 ppb test procedure and continue with the test.

Note: *The lead acetate must not touch the test strip!*

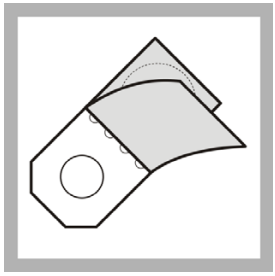
Always wear gloves or wash hands thoroughly after handling lead acetate.

Section 6 Pesticide/Nerve Agent Test

6.1 Pesticide/Nerve Agent procedure



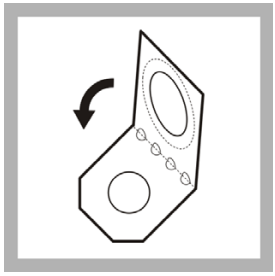
1. Remove one pesticide strip from the storage case. Open the foil packet on the notched side. Remove the contents. Keep the strip and the foil, but put the wadding in the bag.



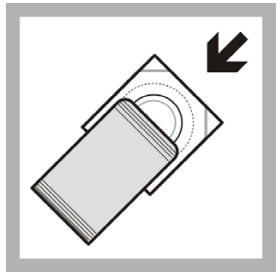
2. The pesticide strip has a white disc at one end and a larger pink disc at the other end. Fold back the protective film covering the white disc only.

3. Put the white disc in the beaker that contains the sample water for at least one minute.

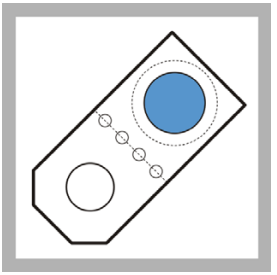
4. Remove the pesticide strip from the sample beaker.



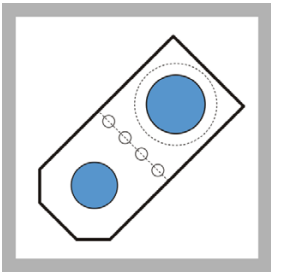
5. Remove the protective film covering the pink disc. Fold the strip in half along the perforations and push the white disc against the pink disc.



6. Put the strip in the pesticide clip and put the strip/clip back into the foil packet. Keep the foil packet warm by holding it under the armpit (outside of clothes) for three to four minutes.



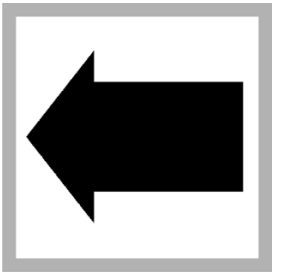
7. Open the strip and look at the color of the smaller disc. For the best results, hold the strip against something white so the color development is easier to see.



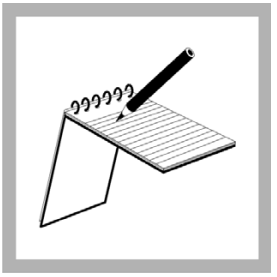
8. There are two possible results:

A white disc indicates POSTIVE–pesticides or nerve agent are present.

A blue disc (matching blue or darker blue than larger disc) indicates NEGATIVE–no pesticide or nerve agent present.



9. Do the test again if a positive result is seen or compare with a test from a known clean water sample.



10. Record the results.

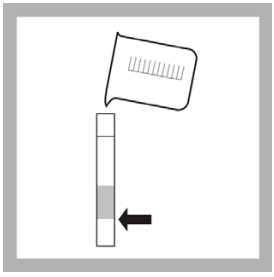
Section 7 Free and Total Chlorine Test

7.1 Test preparation

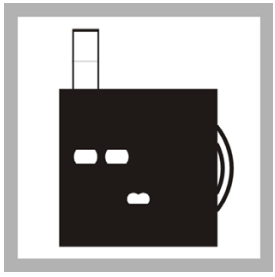
Important Note: This product has not been evaluated to test for chlorine and chloramines in medical applications in the United States.

- Analyze samples immediately after collection.
- Put the color disc on the center pin in the color comparator box (numbers to the front).
- Use sunlight or a lamp as a light source to find the color match with the color comparator box.
- Rinse the tubes with sample before the test. Rinse the tubes with deionized water after the test.
- If the color match is between two segments, use the value that is in the middle of the two segments.
- If the color disc becomes wet internally, pull apart the flat plastic sides to open the color disc. Remove the thin inner disc. Dry all parts with a soft cloth. Assemble when fully dry.
- Undissolved reagent does not have an effect on test accuracy.
- For free chlorine, read the result immediately after the reagent is added to prevent interference from monochloramine. If the sample contains 3.0 mg/L monochloramine, the free chlorine result increases each minute by 0.1 mg/L.

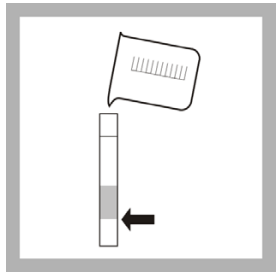
7.2 Free or total chlorine procedure, 0–3.4 mg/L Cl_2



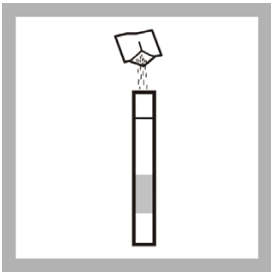
1. Fill a tube to the first line (5 mL) with sample. This is the blank.



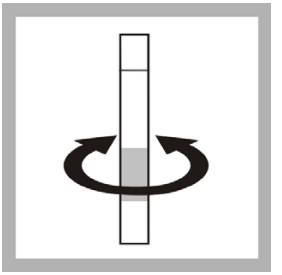
2. Put the tube into the left opening of the color comparator box.



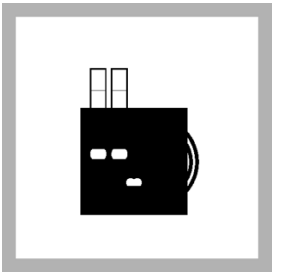
3. Fill another tube to the first line (5-mL) with sample.



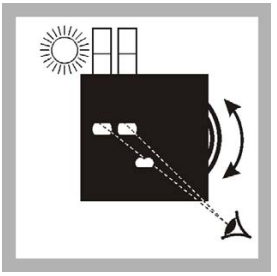
4. Add one DPD (Free or Total) Chlorine Powder Pillow to the second tube.



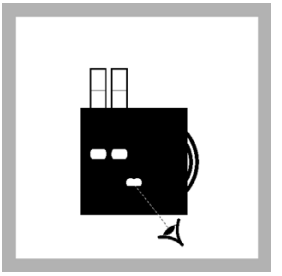
5. Swirl to mix. A pink color develops.
For free chlorine, read the result within 1 minute.
For total chlorine, wait 3 minutes. Read the result within 6 minutes.



6. Put the second tube into the color comparator box.



7. Hold the color comparator box in front of a light source. Turn the color disc to find the color match.



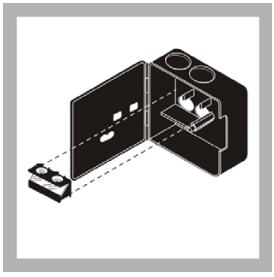
8. Read the result in mg/L in the scale window.

Section 8 Color Test

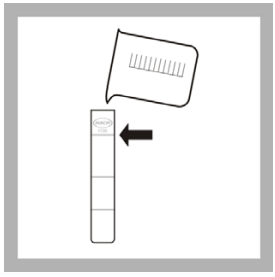
8.1 Test preparation

- Put the color disc on the center pin in the color comparator box (numbers to the front).
- Use sunlight or a lamp as a light source to find the color match with the color comparator box.
- Rinse the tubes with sample before the test. Rinse the tubes with deionized water after the test.
- If the color match is between two segments, use the value that is in the middle of the two segments.
- If the color disc becomes wet internally, pull apart the flat plastic sides to open the color disc. Remove the thin inner disc. Dry all parts with a soft cloth. Assemble when fully dry.
- The long-path adapter for the low range test shows the color in the tubes from top to bottom. Make sure the light source is above the tubes during the color match.

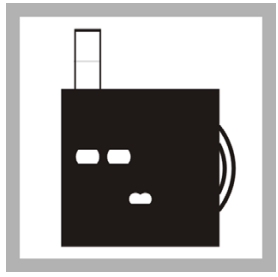
8.2 Color, apparent (0–100 APHA platinum cobalt color units)



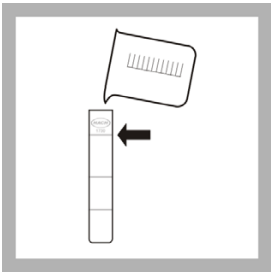
1. Install the longpath adapter in the color comparator box.



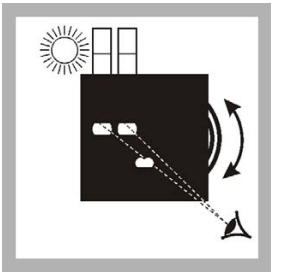
2. Fill a tube to the top line with deionized water or water that has no color.



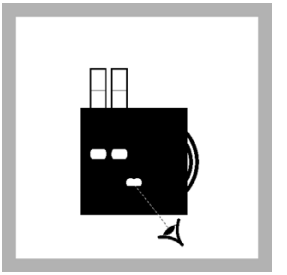
3. Put the tube into left opening of the color comparator box.



4. Fill a second tube to the top line with sample.
Put the second tube into the color comparator box.

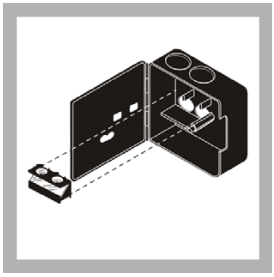


5. Hold the color comparator box below a light source. Turn the color disc to find the color match.

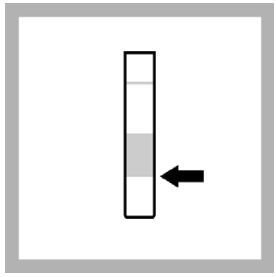


6. Read the results in platinum cobalt color units in the scale window.

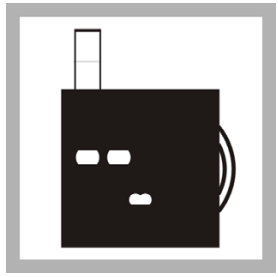
8.3 Color, apparent (0–500 APHA platinum cobalt color units)



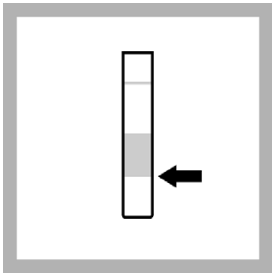
1. If installed, remove the longpath adapter.



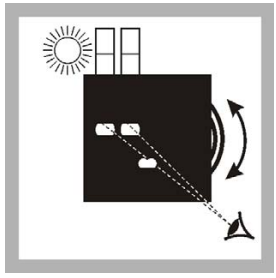
2. Fill a tube to the first line (5 mL) with deionized water or water that has no color.



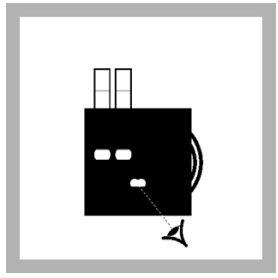
3. Put the tube into the left opening of the color comparator box.



4. Fill a second tube to the first line (5-mL) with sample. Put the second tube into the color comparator box.

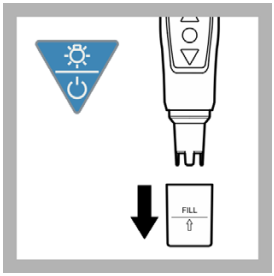


5. Hold the color comparator box in front of a light source. Turn the color disc to find the color match.

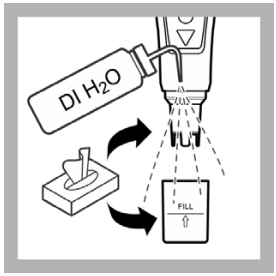


6. Read the value in the scale window.
Multiply the value by 5 to get the result in platinum cobalt color units.

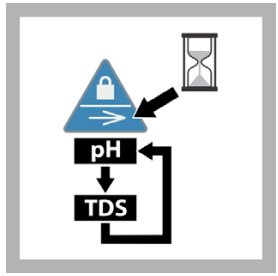
9.1 pH or TDS measurement



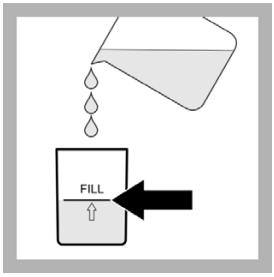
1. Push the Power/Backlight key to set the power to on. Remove the sensor cap.



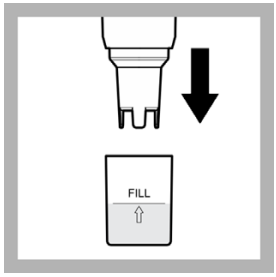
2. Rinse the sensor and the sensor cap with deionized water. Dry with a no-lint cloth.



3. Push and hold the Lock/Parameter key to select pH or TDS as the measurement mode.

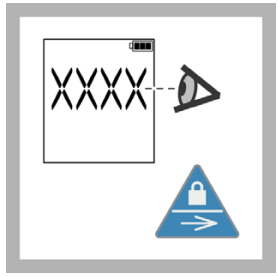


4. Add the sample to the fill line of the sensor cap.



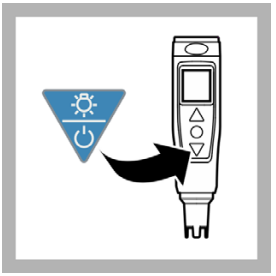
5. Put the sensor fully into the sensor cap. The tester reads the value.

Air bubbles under the probe tip when submerged can cause slow stabilization or error in measurement. Shake the tester from side to side to remove air bubbles.

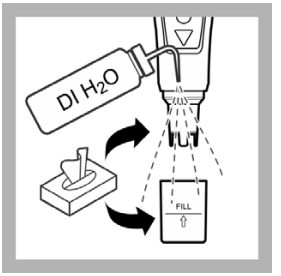


6. When the display is stable, read the value.

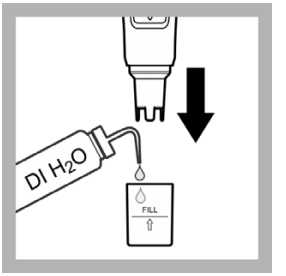
To stabilize and keep the value, push the Lock/Parameter key.



7. Push the Power/Backlight key to set the power to off.



8. Rinse the sensor and the sensor cap with deionized water. Dry with a no-lint cloth.

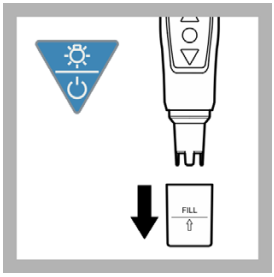


9. For faster response and longer test life, put several drops of deionized water in the sensor cap. This keeps the glass bulb from becoming dry. Put the sensor cap on the sensor.

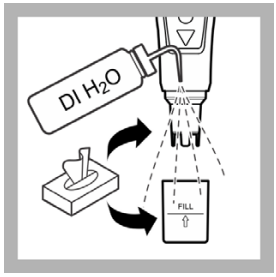
Note: *To extend electrode life, soak the electrode tip in tap water for a few minutes each week.*

Note: *Refer to the documentation supplied with the Pocket Pro+ Multi 2 Tester to do conductivity, salinity and temperature procedures.*

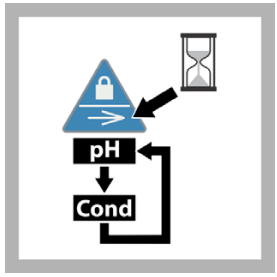
9.2 pH calibration



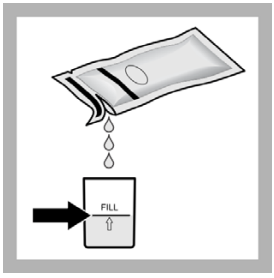
1. Push the Power/Backlight key to set the power to on. Remove the sensor cap.



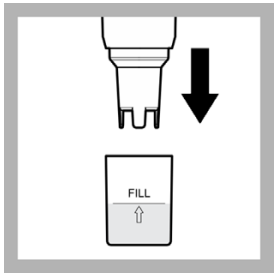
2. Rinse the sensor and the sensor cap with deionized water. Dry with a no-lint cloth.



3. Push and hold the Lock/Parameter key until “pH” shows as the measurement mode.



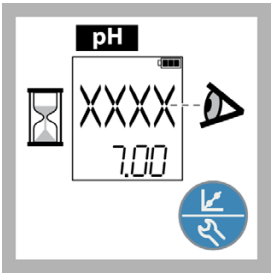
4. Pour a pH 7.00 buffer to the fill line of the sensor cap.



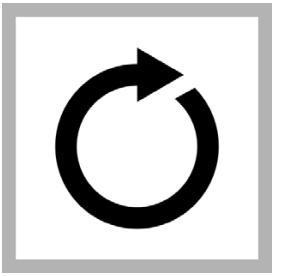
5. Put the sensor fully into the sensor cap. The tester reads the buffer value.

Air bubbles under the probe tip when submerged can cause slow stabilization or error in measurement. Shake the tester from side to side to remove air bubbles.

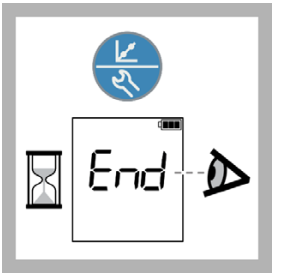
6. Push the Calibration/Settings key to start the calibration.



7. Wait for the value to stabilize, then push the Calibration/Settings key to save the buffer value.

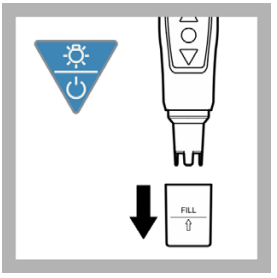


8. Optional: Do steps 2 through 7 againto measure a pH 4.00 and/or a pH 10.00 buffer.

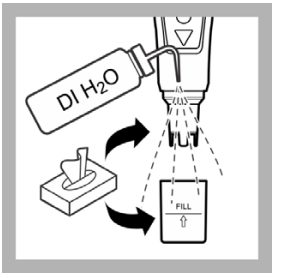


9. Push and hold the Calibration/Settings key to exit.

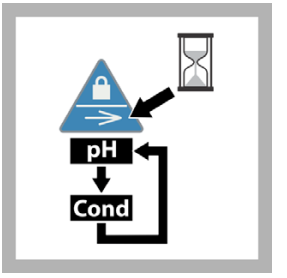
9.3 TDS/Conductivity calibration



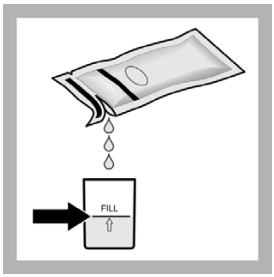
1. Push the Power/Backlight key to set the power to on. Remove the sensor cap.



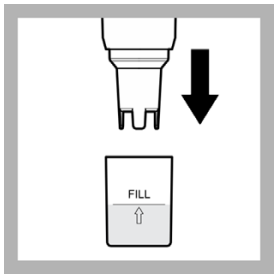
2. Rinse the sensor and the sensor cap with deionized water. Dry with a no-lint cloth.



3. Push and hold the Lock/Parameter key until “Cond” shows as the measurement mode.

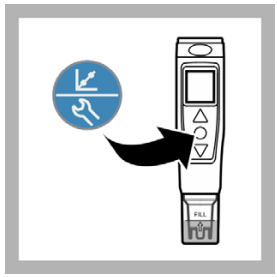


4. Pour a 1413 µS/cm conductivity standard to the fill line on the sensor cap.

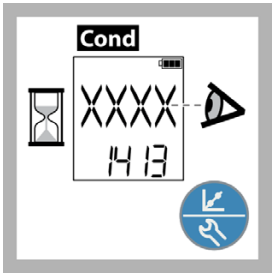


5. Put the sensor fully into the sensor cap. The tester will read the standard value.

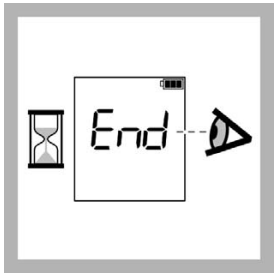
Air bubbles under the probe tip when submerged can cause slow stabilization or error in measurement. Shake the tester from side to side to remove air bubbles.



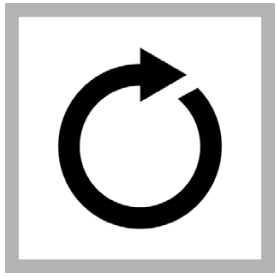
6. Push the Calibration/Settings key to start the calibration.



7. Wait for the value to stabilize, then push the Calibration/Settings key to keep the standard value.



8. The display shows “END” when the calibration is complete.



9. To measure a second standard (147 µS/cm), do step 2, then steps 4 through 7 again.

Section 10 Luminescent Bacteria Toxicity Test: Screening/LIMIT measure

The Luminescent Bacteria Toxicity (LBT) Test screening and LIMIT measure procedures use the luminometer. Before doing either procedure:

- Read section 3.1, Overview on page 15.
- Do the procedures in section 3.2, Prepare the luminometer for use on page 16.
- Print color copies of the Screening Luminescence Results Sheet to use in the field (refer to page 68) from www.Hach.com.

This chapter describes the LBT screening luminescence procedure and LIMIT measure procedure and contains the procedure steps.

The screening luminescence procedure and LIMIT measure procedure are used in the field or in an emergency situation when a rapid assessment of the inhibitory effects of a sample is necessary. The screening luminescence procedure and LIMIT measure procedure are a simplified test procedure that uses the same reagents according to ISO 11348 but at ambient conditions.

The LBT screening luminescence procedure and LIMIT measure procedure are done the same with two exceptions:

- Different options on the luminometer are selected to measure the sample dilutions.
- Different options on the luminometer are selected to show or send results.
- A LIMIT value is set by the user using the luminometer for the LIMIT measure procedure.

A column is added to the LIMIT measure test results that shows whether the percent inhibition measured for each sample dilution is above or below the LIMIT value (percent inhibition) set by the user on the luminometer.

Do the LBT screening luminescence procedure to do a toxicity screening measure. Do the LBT LIMIT measure procedure to do a toxicity limit measure.

10.1 Overview

The screening luminescence procedure or LIMIT measure procedure is done to identify if a sample is free of any inhibitory effects on the luminescent bacteria or, if an inhibition is expected, to make an inhibitory or risk assessment of the sample. Therefore, the user should measure dilutions of a sample and the percent inhibition of the dilution steps to get more information about the severity of the inhibitory effects.

In one run, the sample is measured in three different concentrations: 20% sample, 50% sample and 80% sample. The inhibitory effect of each sample dilution on the

luminescent bacteria is measured by the luminometer and is shown as percent inhibition.

Due to the nature of the simplified procedure and because the test is done at ambient temperatures, the results may be different if compared directly with results for the same sample using the LBT measurement luminescence (ISO 11348) procedure.

10.2 Accuracy

The error or standard deviation of the test is the sum of the error introduced to the test by all components, the ambient and all manipulations. The higher the degree of variation, the higher the total error.

A Luminescent Bacteria Toxicity Test done strictly according to ISO 11348 has a better precision (lower CV (coefficient of variation)) than a LBT simplified luminescence screening procedure or LIMIT measure procedure under field conditions.

For screening measurements and LIMIT measurements, the measurement CV is 7% in the middle of the measuring range of 10-90% inhibition. In practice, samples that shows results of +/-15% inhibition in the 80% sample concentration have no affect in the Luminescent Bacteria Toxicity Test.

If higher precision or lower CV is needed, do the LBT measurement luminescence procedure under more controlled conditions in a lab using additional accessories like a LUMIStherm temperature controlled incubator (LTV053).

10.3 Reagent description

The Luminescent Bacteria Toxicity Test reagent contains living luminescent bacteria that have been grown under optimal conditions, harvested and lyophilized (freeze-dried). The reagent is a freeze-dried preparation of a specially selected strain of the marine bacterium Vibrio fischeri (formerly known as Photobacterium phosphoreum, NRRL number B-11177). A vial of reagent contains roughly one hundred million test organisms.

Refer to section Appendix A, Luminescent bacteria risks on page 121 for bacteria risk information.

10.3.1 Quality assurance test

The standards specify that certain validity criteria must be met for the reagent. Accordingly, a test must be done for each batch of bacteria that is prepared in-house or moved in. The quality certificate delivered with each package of luminescent bacteria reagent by HACH-LANGE GmbH guarantees compliance with the stipulated validity criteria.

To make sure that the test operates correctly on site, the user does control measurements with the standard solutions (refer to the ISO standard procedure). The necessary information about standard substances, test concentrations and

sources of supply is contained in the quality certificate that comes with every box of luminescent bacteria reagent.

The standard stock solutions should be prepared with 2% NaCl solution. The pH of the sample should not be adjusted. Prepare the standard solution such that 0.5 mL of standard solution and 0.5 mL of bacteria solution gives the above mentioned final test concentration. Check in duplicate whether those standard tests give 20–80 % inhibition after 30 minutes of exposure time at 15 °C.

10.4 Reagent storage and preservation

The freeze-dried reagent can be kept at -18 °C until the expiration date shown on the package.

Tubes that contain thawed but not reactivated freeze-dried luminescent bacteria can be frozen again and kept on stock.

The reagent can be transported or shipped up to 7 days at no more than 25 °C.

10.5 Prepare the reagent

Prepare the Luminescent Bacteria Toxicity Test reagent in the field using the procedure in this section.

The amount of light made by the luminescent bacteria is affected by the temperature at which the reagent is reconstituted. The luminescent bacteria and reconstitution solution must be mixed as cold as possible at refrigerator temperatures (3 to 8 °C). If the temperature is higher, the amount of initial light made by the bacteria will be lower.

10.6 Prepare the stock suspension using the LCK491 reagent

Prepare the stock suspension by adding the reconstitution solution to the freeze-fried bacteria reagent. The reconstitution solution rehydrates the bacteria reagent.

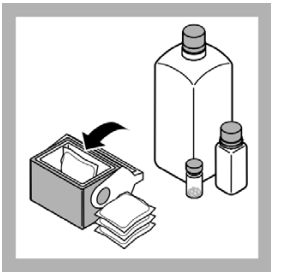
Reconstitution solution is specially made non-toxic ultra pure water. Do not make reconstitution solution or use substitutes.

The dry reagent can be kept at ambient temperatures (not higher than 25 °C) up to 5 days without cooling. Make sure that reactivation conditions are as cool as possible.

The stock suspension can be kept in a refrigerator as long as the validity criteria are met (typically up to 4 hours).

This procedure is temperature sensitive.

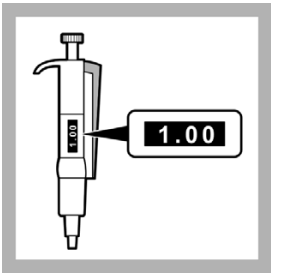
1. Remove the luminescent bacteria test reagent from the freezer. Remove the reconstitution solution and Diluent from refrigerator.



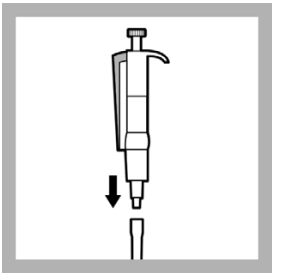
2. Put the frozen luminescent bacteria reagent, refrigerated reconstitution solution and Diluent in a cool box that contains thermal packs if possible.

3. In the field, remove the cap from the reconstitution solution bottle.

4. Remove the foil seal and rubber stopper from the reagent bottle.

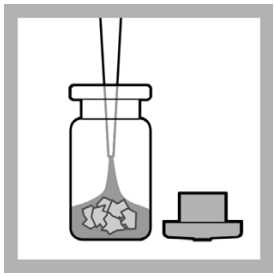


5. Set the 1.0-5.0 mL pipette to 1.0 mL.

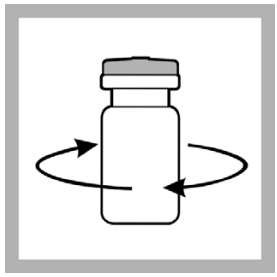


6. Put the end of the 1.0-5.0 mL pipette into a clean pipette tip.

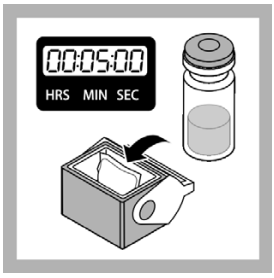
7. Put the tip of the pipette into the reconstitution solution and slowly pull in 1.0 mL.



8. Put the tip of the pipette into the luminescent bacteria reagent bottle and slowly dispense the solution into the reagent.



9. Put the rubber stopper in the reagent bottle. Swirl the reagent bottle to mix.



10. Cool the reagent for 5 minutes in the cool box.

10.7 Prepare the test suspension

Prepare the test suspension (stock suspension and Diluent mixture) by doing the procedure in this section.

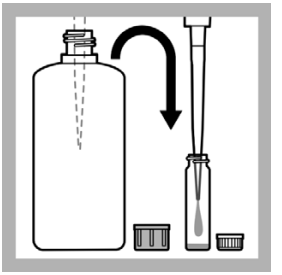
The Diluent is made according to ISO11348-3 and makes sure that the test is not negatively affected by the presence of potassium (K+) and magnesia (Mg2+) ions in the sample. The Diluent is a specially made non-toxic 2% sodium chloride (NaCl) solution that contains potassium and magnesia ions.

The marine bacterium in the reagent requires the osmotic protection that is given by the 2% NaCl in the Diluent. The potassium and magnesium in the Diluent stabilize the light made over time. This stabilization helps keep high negative inhibitions from getting with samples that contain potassium and magnesium ions.

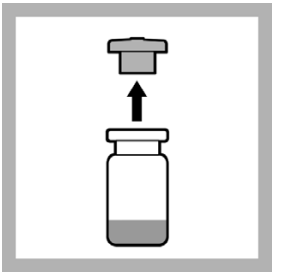
Do not make Diluent or use substitutes.

1. Remove the Diluent from the cool box.

Remove the cap from the Diluent bottle.

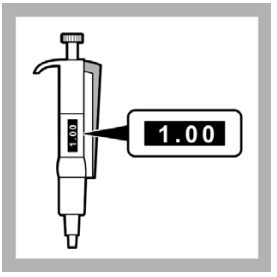


2. Put 14.0 mL of Diluent at refrigerator temperature in the reaction vessel using the pipette.

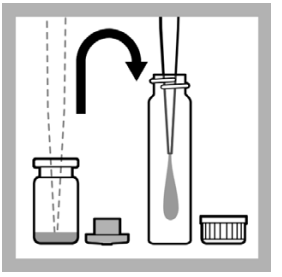


3. Remove the stock suspension (rehydrated reagent) from the cool box.

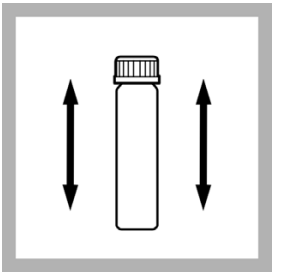
Remove the rubber stopper from the reagent bottle.



4. Set the 1.0-5.0 mL pipette to 1.0 mL.

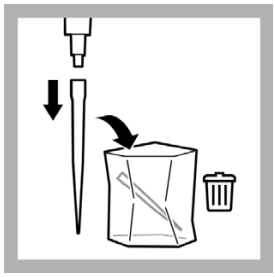


5. Put 1 mL of stock suspension at refrigerator temperature into a clean reaction vessel using the pipette.



6. Put the cap on the reaction vessel and shake to mix thoroughly.

7. Wait 15 minutes.



8. Remove the pipette tip from the pipette and put in the waste bag.

Put the pipette in the storage case.

10.8 Sample collection, storage and preservation

The test can be used with samples of municipal and industrial waste water, aqueous eluates from soil and waste, aqueous solutions of pure chemicals and with surface, well and water of other sources.

Collect samples in clean glass bottles.

Keep samples in the dark at 0 to 5 °C for no longer than 2 days.

Freeze and store samples at -18 °C for not longer than to 2 months. Record preservation activities.

Before use, defrost samples completely. Homogenize the defrosted samples.

10.9 Interferences

Samples interferences can inhibit the light made by luminescent bacteria.

Interfering substances	Interference levels and treatments
Chlorine	Changes the viability of the bacterial reagent. Chlorine is toxic to the bacteria. To remove chlorine from a sample, add one powder pillow of sodium thiosulfate (Hach 1436369 dechlorination agent) to 20 mL of sample and wait for 10 minutes.
High oxygen consumption	Causes light inhibition that is not caused by toxicity
pH	pH-related light inhibition may occur if the pH is below 6.0 or above 8.0. The pH of the sample must be within 7 +/- 0.2 pH units of the standard.

Interfering substances	Interference levels and treatments
Sodium chloride	A sodium chlorine (NaCl) concentrations of less than 15 g/L or more than 50 g/L (or their osmolarity equivalents) in a sample will cause osmosis-related light inhibition. The addition of solid NaCl to the sample (2% final concentration), prevents osmosis-related light inhibition of samples of low or unknown NaCl concentrations.
Temperature	This biological test is strongly temperature-dependent. ISO 11348 requires that the test is done under temperature controlled conditions at 15 °C using a appropriate thermostat (i.e. LUMIStherm, LTV053).
Turbidity and color	Causes high-bias results due to physical absorption or scattering of light. Use color correction cuvettes (accessories) in a separate test according to ISO 11348 or dilute the samples (i.e. 25% or 50%) before testing in the screening measure to remove the interference.

10.10 Prepare the sample

To prepare the sample for testing:

1. If the sample is turbid, either:

- Filter the sample with a modified polysulfone filter

  Before using other filter materials, test the filter material with 2% NaCl first to make sure that the filter material can be used with the Luminescent Bacteria Toxicity Test. Check the acceptable filters in the ISO method.

Note: *Do not use a cellulose nitrate or a cellulose acetate filter. The use of cellulose nitrate or cellulose acetate filters can cause light inhibition that is not caused by the sample.*

- Let the sample sediment for 1 hour, or
- Centrifuge the sample (e.g., 10 minutes at 5.000 g)

2. Check the pH level. Adjust the sample to pH 6 to 8 using HCl or NaOH. Use a strength of HCl or NaOH that does not change the volume of the sample by more than 5% intotal.

3. Add one spoon of solid NaCl (LCX058) and dissolve it in 7 mL of sample. The concentration of salt in the test should not exceed 35 g/L.

Note: *Do not add NaCl to the sample if the salt concentration of the sample is more than 20 g/L (guide value: conductivity of 35 mS/cm).*

Note: *The salt content of the sample should not exceed 50 g/L. This corresponds to a conductivity of about 70 mS/cm without taking other conductive compounds into account.*

Solid NaCl is used to change the sample osmolarity to a value that is correct for the marine bacterium used in the test.

4. If the sample has a high toxicity, carry out a preliminary dilution of the sample with 2% NaCl solution. Select a preliminary dilution from the levels 1:2, 1:4, 1:8, 1:16, etc. to make sure of a continuous dilution series using the dilution procedure of the manufacturer.

10.11 Prepare the test tubes

At the end of this procedure, the test tubes contain the percent sample dilutions shown in Figure 3.

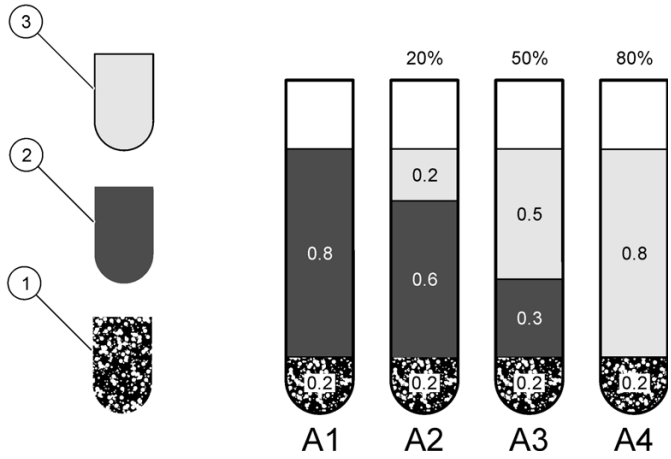
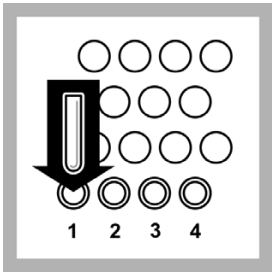
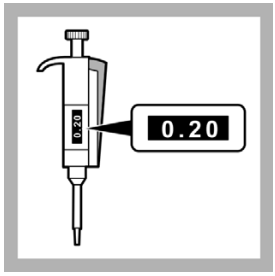


Figure 3 Screening dilution series

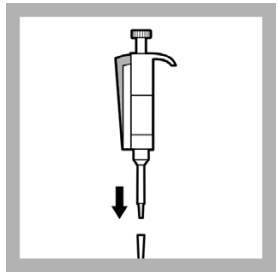
1	Test suspension	**3**	Sample
2	2% NaCl solution		



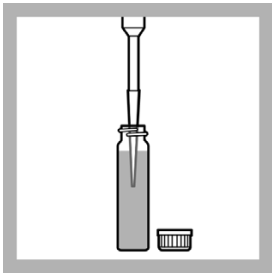
1. Put four test tubes in the test tube stand.



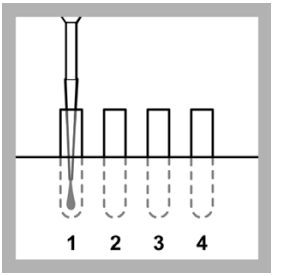
2. Set the 0.2 - 1.0 mL pipette to 0.2 mL.



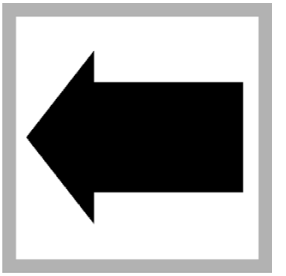
3. Put the end of the pipette into a clean pipette tip.



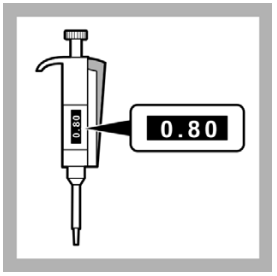
4. Put the tip of the pipette into the test suspension and slowly pull in 0.2 mL.



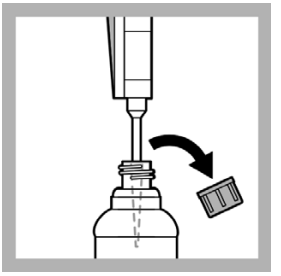
5. Slowly dispense the test suspension into the test tube in position 1.



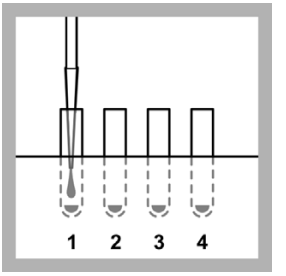
6. Do steps 4 and 5 again until all four test tubes contain 0.2 mL of test suspension



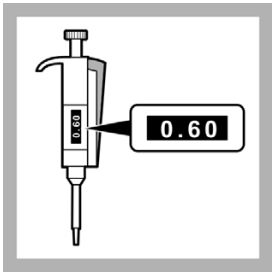
7. Set the 0.2 - 1.0 mL pipette to 0.8 mL.



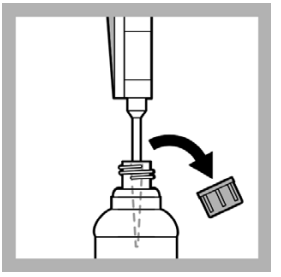
8. Put the tip of the pipette into the 2% NaCl and slowly pull in 0.8 mL.



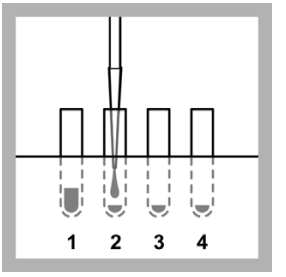
9. Slowly dispense the 2% NaCl solution into the test tube in position 1.



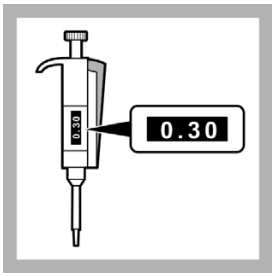
10. Set the 0.2 - 1.0 mL pipette to 0.6 mL.



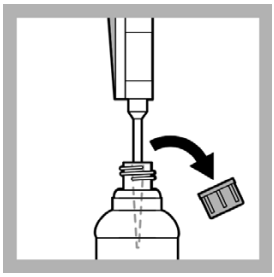
11. Put the tip of the pipette into the 2% NaCl and slowly pull in 0.6 mL.



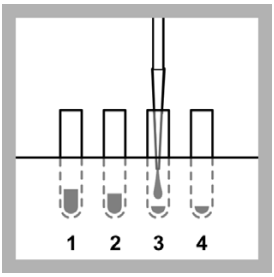
12. Slowly dispense the 2% NaCl solution into the test tube in position 2.



13. Set the 0.2 - 1.0 mL pipette to 0.3 mL.

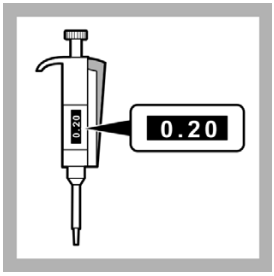


14. Put the tip of the pipette into the 2% NaCl and slowly pull in 0.3 mL.



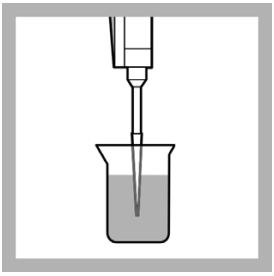
15. Slowly dispense the 2% NaCl solution into the test tube in position 3.

Note: *No 2% NaCl is put in the test tube in position 4.*

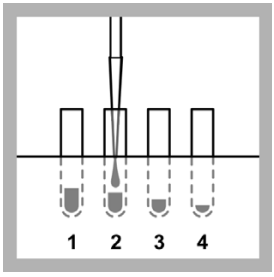


16. Set the 0.2 - 1.0 mL pipette to 0.2 mL.

17. Set the timer clock for 15 minutes (contact time).

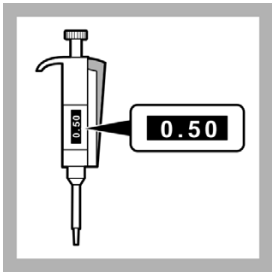


18. Put the tip of the pipette into the sample and slowly pull in 0.2 mL.

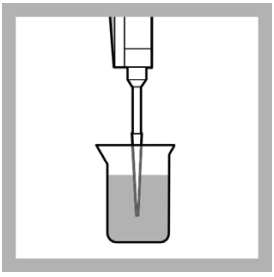


19. Slowly dispense the sample into the test tube in position 2. Start the timer.

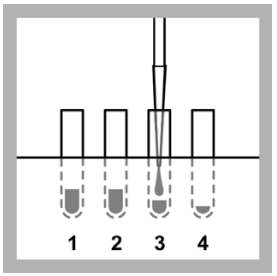
Note: *No sample is put into the test tube in position 1. Test tube 1 is the non-toxic reference.*



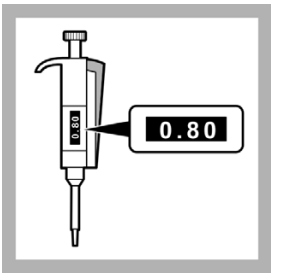
20. Set the 0.2 - 1.0 mL pipette to 0.5 mL.



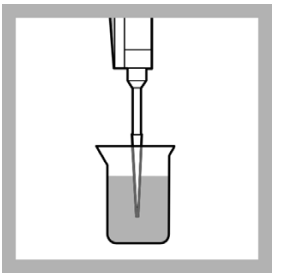
21. Put the tip of the pipette into the sample and slowly pull in 0.5 mL.



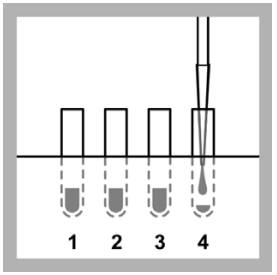
22. Slowly dispense the sample into the test tube in position 3.



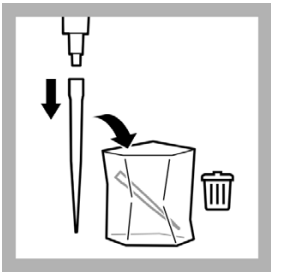
23. Set the 0.2 - 1.0 mL pipette to 0.8 mL.



24. Put the tip of the pipette into the sample and slowly pull in 0.8 mL.



25. Slowly dispense the sample into the test tube in position 4.



26. Remove the pipette tip from the pipette and put in the waste bag.

Put the pipette in the storage case.

10.12 Measure the toxicity of the sample dilutions

The Luminescent Bacteria Toxicity Test is a biological test method and the result is therefore strongly temperature-dependent. Record the temperature at which the test was done. The results of tests done at different temperatures cannot be compared directly.

A non-toxic reference is added to the test suspension during the test and measured. The reference measurement is used to compensate for changes in light levels from the luminescent bacteria. The light levels change with time.

In some instances, if reconstitution is done at the optimum temperature and the test is carried out at 20 °C, the initial light made by the bacteria can be more than 1000 Eclox light units. This causes the error Detector Overload. If an error occurs, change the measurement range from the 0–1000 range to the 0–2000 range and do the readings again (refer to Set the measurement range on page 18).

To measure the toxicity of the sample dilutions:

1. Push **ON** (green button) for several seconds to apply power to the luminometer.

   When the built-in tests are done, push **PROCEED**. The Main Menu is shown.

2. Select Luminescent Bacteria Test and push **ENTER**.

3. Select Measure and push **ENTER**.

4. To do the screening luminescence procedure:

   a. Select Screening Luminescence and push **ENTER**.

   b. Select one option that is shown:

- To measure luminescence and save the results on the luminometer, select Screen and Save and push **ENTER**.
- To measure luminescence and manually record the measuring values on paper, select Screen without Saving and push **ENTER**.
- To measure the luminescence and send the results to a PC, start LUMISsoft on the computer, start the test on LUMISsoft, and when Please select LSoft at the luminometer is shown, select Measure Luminescence and Send to PC and push **ENTER**. The luminometer must be connected to a computer (refer to Connect the luminometer to a computer on page 23).
- To measure the luminescence and print the results on a printer, select Screening and Send to Printer and push **ENTER**. The luminometer must be connected to a printer (refer to Connect the luminometer to a printer on page 21).

5. To do the LIMIT measure procedure:

   a. Select LIMIT Measure and push **ENTER**.

   b. Select Set LIMIT Value and push **ENTER**.

   c. Push **CHANGE** to set the LIMIT value.

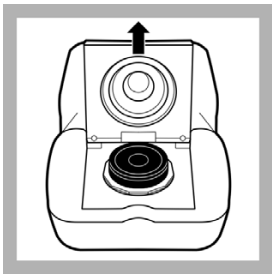
   d. Push **STORE** to save the LIMIT value shown.

   e. Select one option that is shown:

- To measure luminescence and save the results on the luminometer, select LIMIT Measure and Save and push **ENTER**.
- To measure luminescence and manually record the measuring values on paper, select LIMIT Measure without Saving and push **ENTER**.
- To measure the luminescence and send the results to a PC, start LUMISsoft on the computer, start the test on LUMISsoft, and when Please select LSoft at the

Luminometer is shown, select LIMIT Measure and Send to PC and push **ENTER**. The luminometer must be connected to a computer (refer to Connect the luminometer to a computer on page 23).

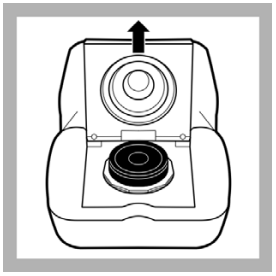
- To measure the luminescence and print the results on a printer, select LIMIT Measure and Send to Printer and push **ENTER**. The luminometer must be connected to a printer (refer to Connect the luminometer to a printer on page 21).



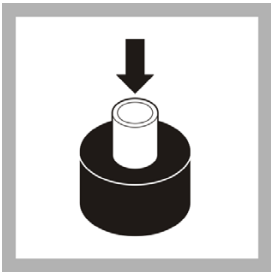
6. Open the luminometer lid and make sure a sample is not in the cell. Close the lid.

7. Push **PROCEED** to show the test status. When the cell tests are done, push **PROCEED** again.

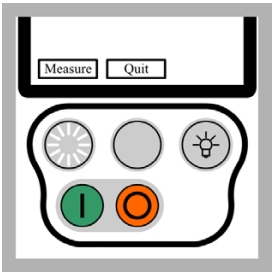
8. Wait until the timer clock completes 15 minutes.



9. Open the luminometer lid.

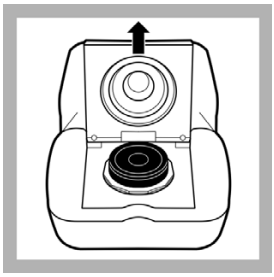


10. Put the test tube in position 1 (non-toxic blank) into the black test tube holder in the luminometer cell.



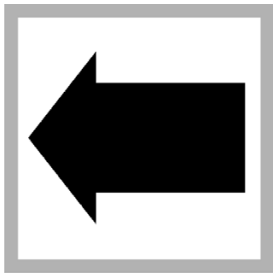
11. Close the luminometer lid. Push **MEASURE**.

When the measurement is complete (approximately 15 seconds), the luminometer shows the relative light units measured.



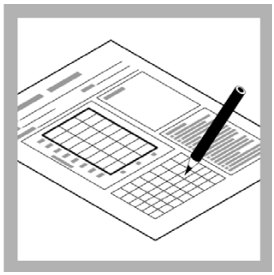
12. Open the lid of the luminometer.

Remove the test tube from the luminometer and put it back in the LUMIStherm.



13. Do steps 22 to 24 again to measure the three other test tubes in the correct order (2, 3, and then 4).

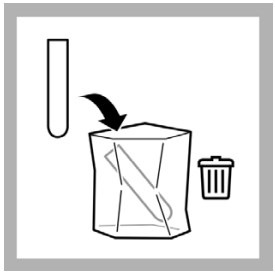
The Inhibit% and rel. units are shown on the screen. Record the Inhibit% and rel. units values for each sample dilution on the Screening Luminescence Results Sheet.



14. Use the color chart on the Screening Luminescence Results Sheet to identify which sample dilutions are toxic (red) and which are non-toxic (green).

Note: *The more of your results in the red zone, the stronger are the inhibitory affects of the sample, the more critical is the sample.*

15. Put the solution in the test tubes into the waste bottle.



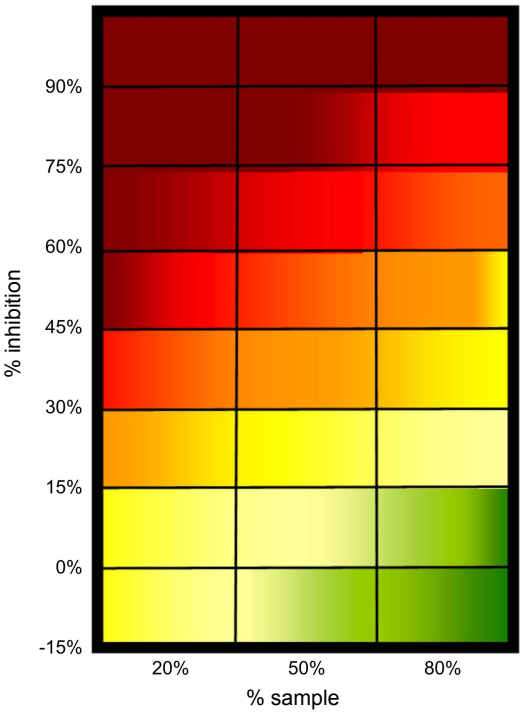
16. Put the test tubes into the waste bag.

Luminescent Bacteria Toxicity Test - Screening Luminescence Results

Sample: _____

Date: _____ Time: _____

Operator: _____



Order	1st	2nd	3rd	% inhib.	Rel. units	
Tube	Test susp. (mL)	2% NaCl (mL)	Sample (mL)			Sample Conc.
1	0.2	0.8	no			Non-toxic reference
2	0.2	0.6	0.2			20%
3	0.2	0.3	0.5			50%
4	0.2	no	0.8			80%

Comments:

Procedure:

1. Add 1.0 mL of reconstitution solution to the reagent. Swirl to mix. Wait 5 minutes.
2. Add 1.0 mL of stock suspension to 10 mL of Diluent. Shake to mix. Wait 15 minutes.
3. Add one spoon of solid NaCl to 7 mL of sample.
4. Fill four test tubes with the test suspension, 2% NaCl, and sample in the order shown in the table. Start the timer after adding the first sample volume. Set the timer for 15 minutes.
5. Push **ON** (green button) on the luminometer. Go to the Screening Luminescence Menu.
6. Measure test tube 1 (non-toxic reference).
7. Measure the sample tubes in the order 2, 3 and then 4. Record the results in the table.

10.13 Show or send previous results

To show previous results on the luminometer, do the procedure in this section for the type of procedure done.

To send previous results to a computer:

Note: *At this stage, the results cannot be sent to the LUMISsoft 4.*

1. Do the steps in Connect the luminometer to a computer on page 23.
2. Start LUMISsoft.
3. In LUMISsoft, select Transfer, Options, Interface Protocol, Connect.
4. Do the procedure in this section for the type of procedure done.

To send previous results to a printer, do the steps in Connect the luminometer to a printer on page 21 and then do the procedure in this section for the type of procedure done.

10.13.1 Description of screening luminescence results

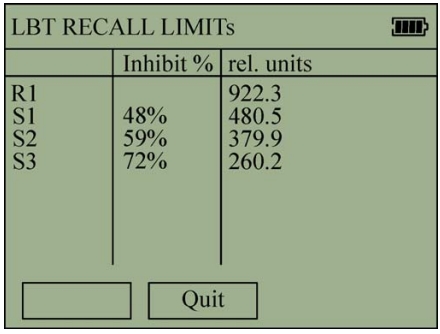
Reference (non-toxic) luminescent measurements are saved as R1 to Rx. The counter starts with R1 every time the storage is erased from the luminometer.

Sample luminescent measurements are done after a reference luminescent measurement is done. Sample luminescent measurements are saved as S1 to Sx.

The luminometer records reference and sample measurements and then calculates the percent inhibition value for each sample measurement (refer to Figure 4).

For example, two different screening luminescence tests have been done. One test with 3 samples or sample dilutions and one test with two samples or sample dilutions. The results of the first test are indicated as R1 with S1,S2 and S3. The results of the second tests are indicated as R2 with S1 and S2.

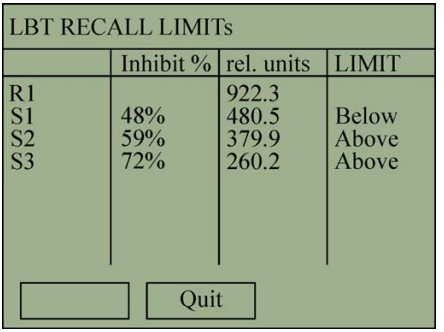
Figure 4 Example of screening luminescence results



10.13.2 Description of LIMIT measure results

The LIMIT measure procedure results are recorded the same as the screening luminescence results. The only difference is that the LIMIT measure results include a column that shows whether the percentage inhibition calculated for each sample measurement is above the LIMIT value or below the LIMIT value set by the user as shown in Figure 5.

Figure 5 Example LIMIT measure results



10.13.3 Show or send screening luminescence results

To show or send previous results saved on the luminometer for the screening luminescence procedure:

1. Push **ON** to apply power to the luminometer.

   The luminometer does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED**.

   The Main Menu is shown.

3. Select LUMINESCENT BACTERIA TEST and push **ENTER**.

   The Luminescent Bacteria Test Main Menu is shown.

4. Select Previous Results and push **ENTER**.

   The Previous Results Menu is shown.

5. Select Show Previous Screenings and push **ENTER**.

   The Previous Screenings Menu is shown.

6. To show all or send all of the results saved on the luminometer, select one option:

- To show the results on the luminometer, select Show all (R1 to Rx) and push **ENTER**.
- To send the results to the computer, select Send all (R1 and Rx) to PC and push **ENTER**.
- To send the results to the printer, select Send all (R1 to Rx) to Printer and push **ENTER**.

7. To show or send a specific range of results saved on the luminometer, select one option:

- To show the results on the luminometer, select Show selection and push **ENTER**.
- To send the results to the computer, select Send selection to PC and push **ENTER**.
- To send the results to the printer, select Send selection to Printer and push **ENTER**.

8. If an option in step 7 was selected, select the data to be recalled:

a. Select the starting indicator in the From field. Push **SELECT** to change the value. Then push **PROCEED**.

b. Select the ending indicator in the To field. Push **SELECT** to change the value. Then push **SHOW**.

9. Push **PROCEED** to show more results.

10.13.4 Show or send LIMIT measure results

To show or send previous results saved on the luminometer for the LIMIT measure procedure:

1. Push **ON** to apply power to the luminometer.

The luminometer does built-in tests that make sure that the electronics and software are operating correctly.

2. When the built-in tests are done, push **PROCEED**.

The Main Menu is shown.

3. Select LUMINESCENT BACTERIA TEST and push **ENTER**.

The Luminescent Bacteria Test Main Menu is shown.

4. Select Previous Results and push **ENTER**.

The Previous Results Menu is shown.

5. Select Show Previous LIMITs and push **ENTER**.

The Previous LIMITs Menu is shown.

6. To show all or send all of the results saved on the luminometer, select one option:

   - To show the results on the luminometer, select Show all (R1 to Rx) and push **ENTER**.
   - To send the results to the computer, select Send all (R1 and Rx) to PC and push **ENTER**.
   - To send the results to the printer, select Send all (R1 to Rx) to Printer and push **ENTER**.

7. To show or send a specific range of results saved on the luminometer, select one option:

   - To show the results on the luminometer, select Show selection and push **ENTER**.
   - To send the results to the computer, select Send selection to PC and push **ENTER**.
   - To send the results to the printer, select Send selection to Printer and push **ENTER**.

8. If an option in step 7 was selected, select the data to be recalled:

   a. Select the starting value in the From field. Push **SELECT** to change the starting value. Then push **PROCEED**.

   b. Select the ending value in the To field. Push **SELECT** to change the ending value. Then push **SHOW**.

9. Push **PROCEED** to show more results.

Section 11 Luminescent Bacteria Toxicity Test ISO 11348 part 3

The Luminescent Bacteria Toxicity (LBT) Test uses the luminometer. Before doing the procedure, read section 3.1, Overview on page 15 and do the procedures in section 3.2, Prepare the luminometer for use on page 16.

This chapter describes the LBT Test measurement luminescence procedure and contains the procedure steps. Use the LBT Test measurement luminescence procedure if the test needs to be done according to ISO 11348 part 3. The Eclox LBT Test measurement luminescence procedure meets the criteria of validation of ISO 11348-3.

11.1 Overview

The test criterion is luminescence which is measured after a contact time of 15 or 30 minutes (optionally 5 minutes at 15 °C) taking into account a correction factor (fK). The correction factor is a measure of intensity change of control samples during the exposure time (refer to the ISO standard procedure).

The luminometer measurements are in relative light units. The luminometer measurements are used by the LUMISsoft computer program or custom made calculations to calculate percent inhibition, LID, EC20 and EC50 values.

- **Percent inhibition**—the percentage of light made by the bacteria that is inhibited by the sample. The higher the percentage inhibition of the light emission, the more harmful the sample is to the bacteria and the higher the toxicity level of the sample.

- **LID**—first dilution value of a sample that causes less than 20% inhibition. The higher the LID, the more harmful the sample is to the bacteria.

- **EC20** or **EC50**—the concentration of a sample that causes exactly 20 or 50% inhibition. The lower the EC-value, the more harmful the sample is to the bacteria.

The linear measuring range is between 10% and 90% inhibition. Refer to ISO 11348 for more detailed information on the Luminescent Bacteria Toxicity Test.

11.2 Accuracy

The error or standard deviation of the test is the sum of the error introduced to the test by all components, the ambient and all manipulations. The higher the degree of variation, the higher the total error.

A Luminescent Bacteria Toxicity Test done strictly according to ISO 11348 has a better precision (lower CV (coefficient of variation)) than a simplified screening test under field conditions.

The total error for the test is typically lower than 20%.

11.3 Thermostat and PC software requirements

ISO 11348 states that the measuring luminometer must have a 15 °C temperature controlled measuring well. The Eclox does not have a temperature controlled measuring cell.

According to ISO 11348 optional accessories that should be used in the lab include:

- LTV053 LUMIStherm, 230V, thermostat to 15 °C
- LZV093 LUMISsoft 4 PC software

11.4 Reagent description

The Luminescent Bacteria Toxicity Test reagent contains living luminescent bacteria that have been grown under optimal conditions, harvested and lyophilized (freeze-dried). The reagent is a freeze-dried preparation of a specially selected strain of the marine bacterium Vibrio fischeri (formerly known as Photobacterium phosphoreum, NRRL number B-11177). A vial of reagent contains roughly one hundred million test organisms.

Refer to section Appendix A, Luminescent bacteria risks on page 121 for bacteria risk information.

The standards stipulate that certain validity criteria must be complied with for the reagent. Accordingly, a test is done for each batch of bacteria that is prepared in-house or bought in. The quality certificate delivered with each package of luminescent bacteria reagent by HACH-LANGE GmbH guarantees compliance with the stipulated validity criteria.

To make sure that the test operates correctly on site, do control measurements with the standard solutions (refer to the ISO standard procedure). The necessary information about standard substances, test concentrations and sources of supply is contained in the quality certificate that comes with every box of luminescent bacteria reagent.

11.5 Reagent storage and preservation

The freeze-dried reagent can be kept at -18 °C until the expiration date shown on the package.

Tubes that contain thawed but not reactivated freeze-dried luminescent bacteria can be frozen again and kept on stock.

The reagent can be transported or shipped up to 7 days at no more than 25 °C.

11.6 Prepare the reagent

Prepare the Luminescent Bacteria Toxicity Test reagent not more than 4 hours before testing according to ISO 11348 as done in this section.

The amount of light made by the luminescent bacteria is affected by the temperature at which the reagent is reconstituted. The luminescent bacteria and reconstitution solution must be mixed as cold as possible at refrigerator temperatures (3 to 8 °C). If the temperature is higher, the amount of initial light made by the bacteria will be lower.

11.6.1 Prepare the stock suspension using the LCK491 reagent

Prepare the stock suspension by adding the reconstitution solution to the freeze-fried bacteria reagent. The reconstitution solution rehydrates the bacteria reagent.

Reconstitution solution is specially made non-toxic ultra pure water. Do not make reconstitution solution or use substitutes.

The stock suspension can be kept in a refrigerator (+8 °C) without being diluted with Diluent as long as the validity criteria are met. Typically up to 4 hours. The sensitivity spectrum of reactivated bacteria may shift as time elapses.

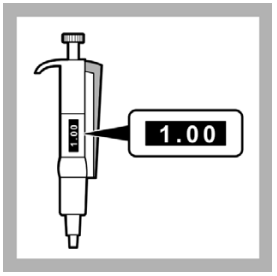
If the reagent is to be used 90 minutes or more after reconstitution, periodically monitor the performance of the reagent with a suitable standard to show changes in sensitivity.

This procedure is temperature sensitive.

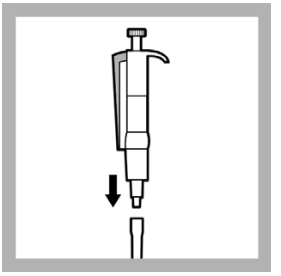
1. Remove the luminescent bacteria test reagent from the freezer. Remove the reconstitution solution from refrigerator.

2. Remove the cap from the reconstitution solution bottle.

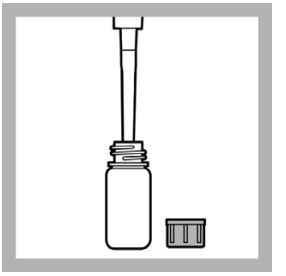
3. Remove the foil seal and rubber stopper from the reagent bottle.



4. Set the 1.0-5.0 mL pipette to 1.0 mL.

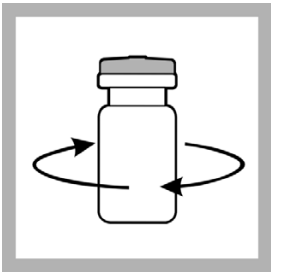


5. Put the end of the pipette into a clean pipette tip.



6. Put the tip of the pipette into the reconstitution solution and slowly pull in 1.0 mL.

7. Put the tip of the pipette into the luminescent bacteria reagent bottle. Quickly dispense the solution into the reagent.



8. Put the rubber stopper in the reagent bottle. Swirl the reagent bottle to mix.

9. Cool the sample for at least 15 minutes in a refrigerator.

11.6.2 Prepare the test suspension

Prepare enough test suspension (stock suspension and Diluent mixture) to do the test. Each test tube used for the test is filled with 0.5 mL of test suspension. To identify the number of test tubes used for a test:

- For D 2 values an higher, add 1 to the number of sample dilution steps to be measured (e.g. 1 blank + 9 dilutions = 10). Then multiply that number by 2.
- For D 1 values and higher, add 2 to the number of sample dilution steps to be measured (2 blanks + 3 dilutions = 5). Then multiply that number by 2.

The Diluent is made according to ISO11348-3 and makes sure that the test is not negatively affected by the presence of potassium (K+) and magnesia (Mg2+) ions in the sample. The Diluent is a specially made non-toxic 2% sodium chloride (NaCl) solution that contains potassium and magnesia ions.

The marine bacterium in the reagent requires the osmotic protection that is given by the 2% NaCl in the Diluent. The potassium and magnesium in the Diluent stabilize the light made over time. This stabilization helps keep high negative inhibitions from getting with samples that contain potassium and magnesium ions.

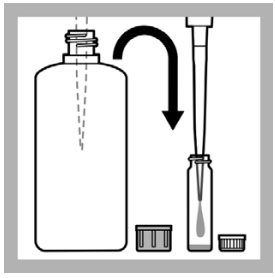
Do not make Diluent or use substitutes.

11.6.2.1 Test suspension for D 2 values and higher

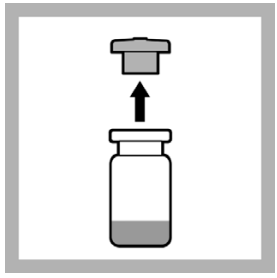
Prepare the test suspension for D2 values and higher if the sample is expected to be toxic.

1. Remove the Diluent from the cool box.

Remove the cap from the Diluent bottle.

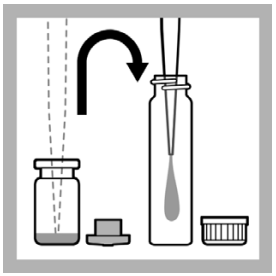


2. Put 50 parts Diluent solution (D) at refrigerator temperature into the reaction vessel using a pipette.



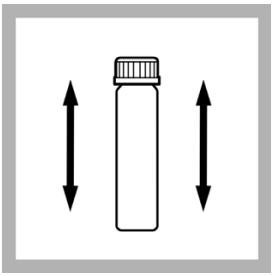
3. Remove the stock suspension (rehydrated reagent) from the cool box.

Remove the rubber stopper from the reagent bottle.

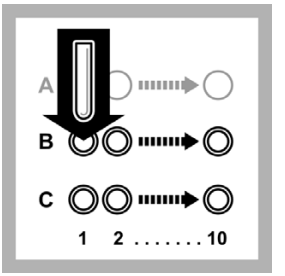


4. Put 1 part stock suspension (S) at refrigerator temperature into a clean reaction vessel using a pipette.

For example:
0.2 mL S + 10 mL D

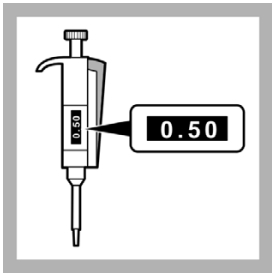


5. Put the cap on the reaction vessel and shake to mix thoroughly.

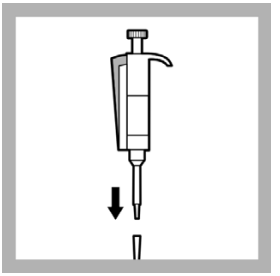


6. Put one half of the new, empty test tubes in Row B and one half of the test tubes in Row C of the LUMIStherm.

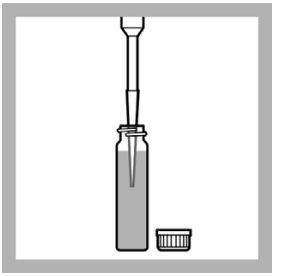
Note: *The LUMIStherm should be operating at 15 °C.*



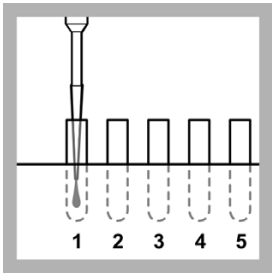
7. Set the 0.2 - 1 mL pipette to 0.5 mL.



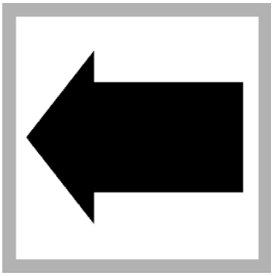
8. Put the end of the pipette into a clean pipette tip.



9. Put the tip of the pipette into the reaction vessel and slowly pull in 0.5 mL of the test suspension.

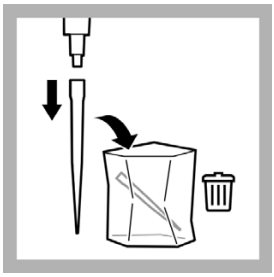


10. Slowly dispense the test suspension into the test tube in position B1.



11. Do step 9 and 10 again until each test tube in Row B and Row C contains 0.5 mL of test suspension.

12. Cool the filled test tubes in the LUMIStherm at 15 °C for 15 minutes.



13. Remove the pipette tips from the pipettes and put in the waste bag.

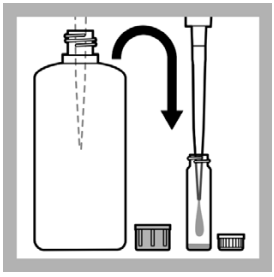
Put the pipettes in the storage case.

11.6.2.2 Test suspension for D 1 values

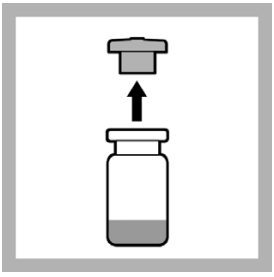
Prepare the test suspension for D1 values if the sample is probably non-toxic to measure the sample toxicity using the highest possible sample concentration of 80% (= D 1).

1. Remove the Diluent from the refrigerator.

Remove the cap from the bottle.

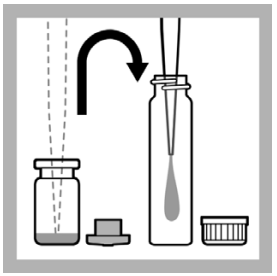


2. Put 20 parts Diluent solution (D) at refrigerator temperature into the reaction vessel using a pipette.



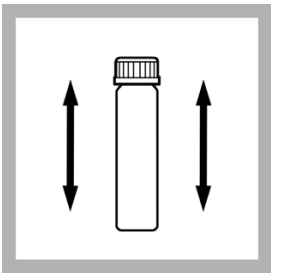
3. Remove the stock solution (rehydrated reagent) from the refrigerator.

Remove the rubber stopper from the reagent bottle.



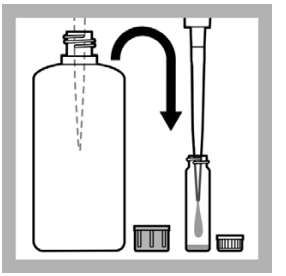
4. Put 1 part stock suspension (S) at refrigerator temperature into a clean reaction vessel using a pipette.

For example:
0.1 mL S + 2.0 mL D

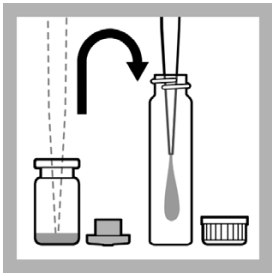


5. Put the cap on the reaction vessel and shake to mix thoroughly.

Put a “1:20” label on the reaction vessel.

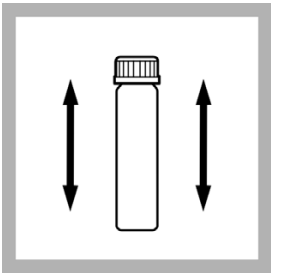


6. Put 50 parts Diluent solution (D) at refrigerator temperature into a clean reaction vessel using a pipette.



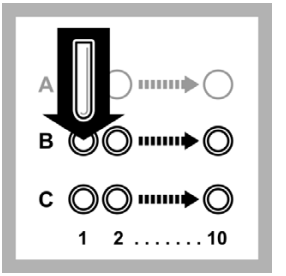
7. Put 1 part stock suspension (S) at refrigerator temperature into a clean reaction vessel using a pipette.

For example:
0.2 mL S + 10 mL D



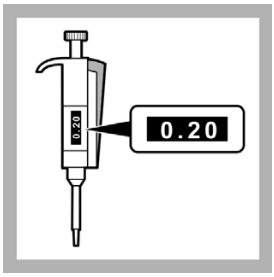
8. Put the cap on the reaction vessel and shake to mix thoroughly.

Put a “1:50” label on the reaction vessel.

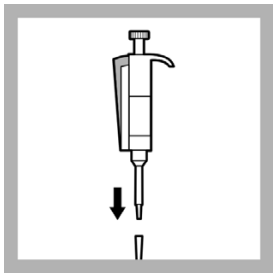


9. Put one half of the new, empty test tubes in Row B and one half of the test tubes in Row C of the LUMIStherm.

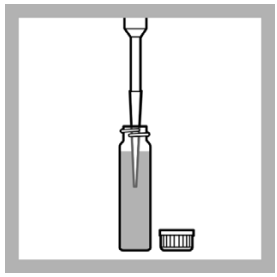
Note: *The LUMIStherm should be operating at 15 °C.*



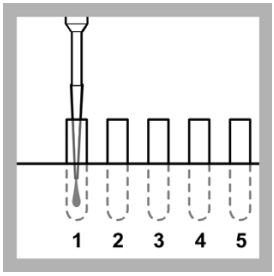
10. Set the 0.2 - 1 mL pipette to 0.2 mL.



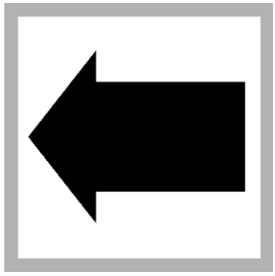
11. Put the end of the pipette into a clean pipette tip.



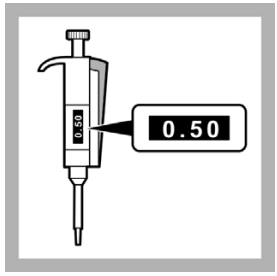
12. Put the tip of the pipette into the reaction vessel that contains the 1:20 test suspension and slowly pull in 0.2 mL of the test suspension.



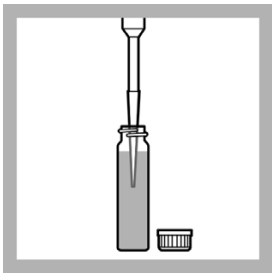
13. Slowly dispense the test suspension into the test tube in position B1.



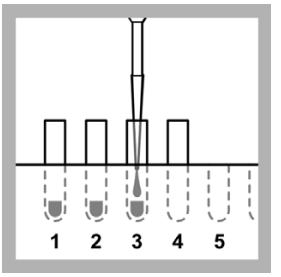
14. Do step 12 and 13 again until the test tubes in position C1, B2 and C2 contain 0.2 mL of test suspension.



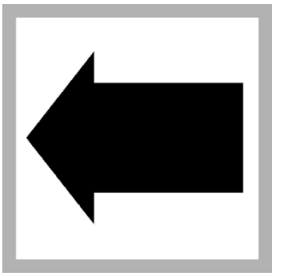
15. Set the 0.2 - 1 mL pipette to 0.5 mL.



16. Put the tip of the pipette into the reaction vessel that contains the 1:50 test suspension and slowly pull in 0.5 mL of the test suspension.

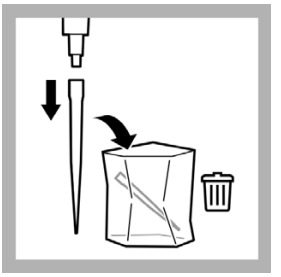


17. Slowly dispense the test suspension into the test tube in position B3.



18. Do step 16 and 17 again until each test tube in Row B and Row C (position 3 and higher) contains 0.5 mL of test suspension.

19. Cool the filled test tubes in the LUMIStherm at 15 °C for 15 minutes.



20. Remove the pipette tips from the pipettes and put in the waste bag.

Put the pipettes in the storage case.

11.7 Sample collection, storage and preservation

The test can be used with samples of municipal and industrial waste water, aqueous eluates from soil and waste, aqueous solutions of pure chemicals and with surface, well and water of other sources.

Collect samples in clean glass bottles.

Keep samples in the dark at 0 to 5 °C for no longer than 2 days.

Freeze and store samples at -18 °C for not longer than to 2 months. Record preservation activities.

Before use, defrost samples completely. Homogenize the defrosted samples.

11.8 Interferences

Samples interferences can inhibit the light made by luminescent bacteria.

Interfering substances	Interference levels and treatments
Chlorine	Affects the viability of the bacterial reagent. Chlorine is toxic to the bacteria. To remove chlorine from a sample, add one powder pillow of sodium thiosulfate (Hach 1436369 dechlorination agent) to 20 mL of sample and wait for 10 minutes.
High oxygen consumption	Causes light inhibition that is not caused by toxicity
pH	pH-related light inhibition may occur if the pH is below 6.0 or above 8.0. The pH of the sample must be within 7 +/- 0.2 pH units of the standard.
Sodium chloride	A sodium chlorine (NaCl) concentrations of less than 15 g/L or more than 50 g/L (or their osmolarity equivalents) in a sample will cause osmosis-related light inhibition. The addition of solid NaCl to the sample (2% final concentration), prevents osmosis-related light inhibition of samples of low or unknown NaCl concentrations.
Temperature	This biological test is strongly temperature-dependent. ISO 11348 requires that the test is done under temperature controlled conditions at 15 °C using a appropriate thermostat (i.e. LUMIStherm, LTV053).
Turbidity and color	Cause high-bias results due to physical absorption or scattering of light. Use color correction cuvettes (accessories) in a separate test according to ISO 11348 or dilute the samples (i.e. 25% or 50%) before testing in the screening measure to remove the interference.

11.9 Prepare the sample

To prepare the sample for testing:

1. If the sample is turbid, either:

   - Filter the sample with a modified polysulfone filter

     Before using other filter materials, test the filter material with 2% NaCl first to make sure that the filter material can be used with the Luminescent Bacteria Toxicity Test. Check the acceptable filters in the ISO method.

Note: *Do not use a cellulose nitrate or a cellulose acetate filter. The use of cellulose nitrate or cellulose acetate filters can cause light inhibition that is not caused by the sample.*

   - Let the sample sediment for 1 hour, or
   - Centrifuge the sample (e.g., 10 minutes at 5.000 g)

2. Check the pH level. Adjust the sample to pH 6 to 8 using HCl or NaOH. Use a strength of HCl or NaOH that does not change the volume of the sample by more than 5% intotal.

3. Add solid NaCl to the sample until the concentration in the sample is 2% (w/v). For example, weigh out 0.3 g of NaCl and dissolve it in 15 mL of sample or dissolve one spoon of solid NaCl (LCX058) in 7 mL of sample. The concentration of salt in the test should not exceed 35 g/L.

Note: *Do not add NaCl to the sample if the salt concentration of the sample is more than 20 g/L (guide value: conductivity of 35 mS/cm).*

Note: *The salt content of the sample should not exceed 50 g/L. This corresponds to a conductivity of about 70 mS/cm without taking other conductive compounds into account.*

   Solid NaCl is used to change the sample osmolarity to a value that is correct for the marine bacterium used in the test.

4. If the sample has a high toxicity, carry out a preliminary dilution of the sample with 2% NaCl solution. Select a preliminary dilution from the levels 1:2, 1:4, 1:8, 1:16, etc. to make sure of a continuous dilution series using the dilution procedure of the manufacturer.

11.10 Prepare the dilutions series

Prepare the sample dilutions series using one of the procedures in this section.

The sample dilutions are added to the test suspension later to identify the percent inhibition of each sample dilution.

A non-toxic reference is added to the test suspension during the test and measured. The reference measurement is used to compensate for changes in light levels from the luminescent bacteria. The light levels change with time.

11.10.1 Prepare a 9 dilution series (D 2 values and higher)

To make a 9 dilution series according to ISO 11348 of D 2 sample values and higher, do this procedure.

This procedure makes dilutions ranging from undiluted to a dilution ratio of 1:16 in Row A. This corresponds to D values of 2 to 32 in the test (Figure 6), as 0.5 mL of the sample dilution is added to 0.5 mL of the test suspension during the test in Row B and C. Adding test suspension to the sample dilutions increases the sample dilutions in row A by a factor of two as final test concentration.

Note: *The test tubes in the higher Row A positions are more concentrated. The pipette is moved from A9 to A2 (higher concentration to lower concentration) when making the dilution series, so the pipette tip does not need to be replaced during this procedure.*

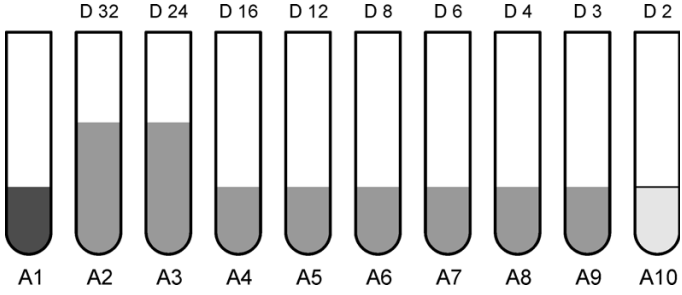
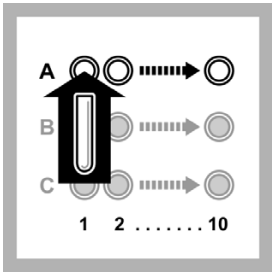
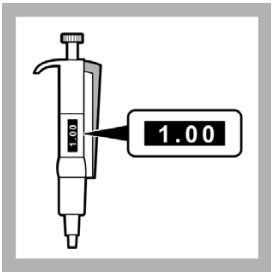


Figure 6 Dilution series - 9 dilutions, D 2 values and higher

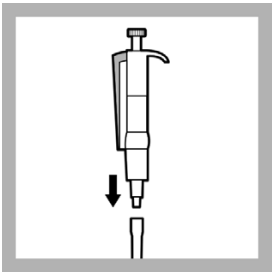
Tube	Contents
A1	2% NaCl (1.5 mL)
A2, A3	2% NaCl and sample (3.0 mL)
A4 - A9	2% NaCl and sample (1.5 mL)
A10	Sample (1.5 mL)



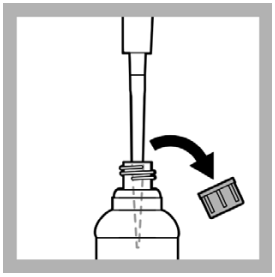
1. Put 10 empty test tubes into Row A of the LUMIStherm.



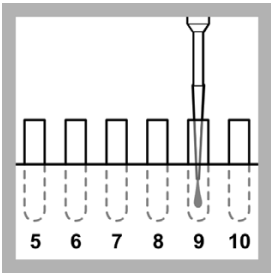
2. Set the 1.0 - 5.0 mL pipette to 1.0 mL.



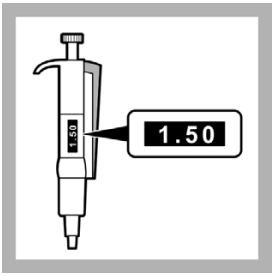
3. Put the end of the pipette into a clean pipette tip.



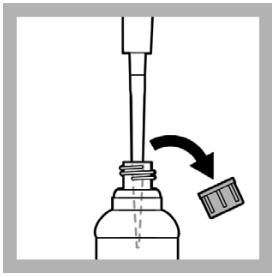
4. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 1.0 mL.



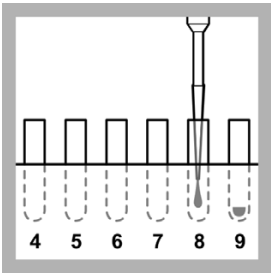
Slowly dispense the 2% NaCl solution into the test tube in position A9.



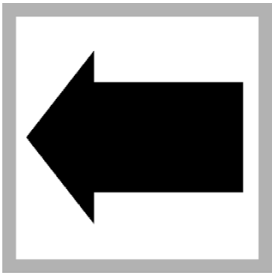
5. Set the 1.0 - 5.0 mL pipette to 1.5 mL.



6. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 1.5 mL.

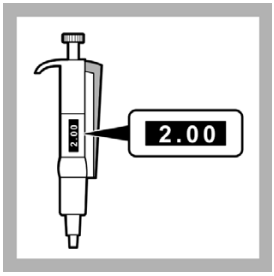


7. Slowly dispense the 2% NaCl solution into the test tube in position A8.

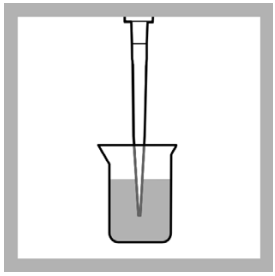


8. Do steps 6 and 7 againto put 1.5 mL of 2% NaCl solution in each test tube in positions A7, A6, A5, A4, A3, A2 and A1.

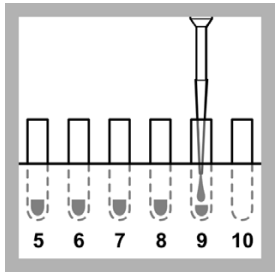
Note: *Do not put 2% NaCl into the test tube in position A10.*



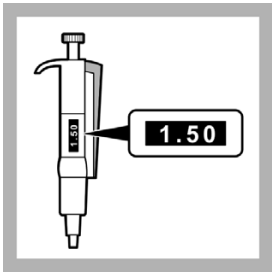
9. Set the 1.0 - 5.0 mL pipette to 2.00 mL.



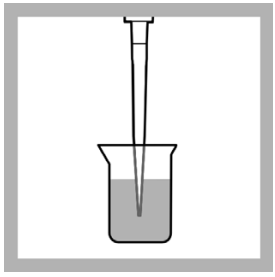
10. Put the tip of the pipette into the sample and slowly pull in 2.0 mL.



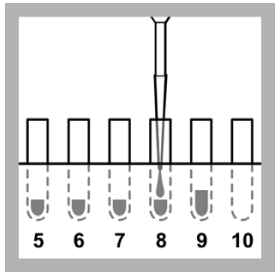
11. Slowly dispense the sample into the test tube in position A9.



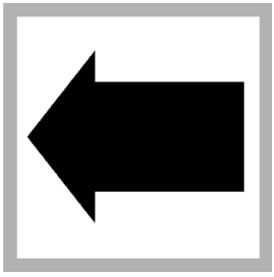
12. Set the 1.0 - 5.0 mL pipette to 1.5 mL.



13. Put the tip of the pipette into the sample and slowly pull in 1.5 mL.

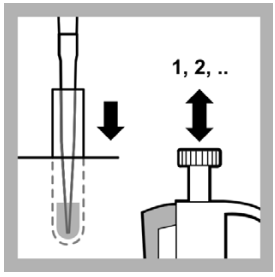


14. Slowly dispense the sample into the test tube in position A8.

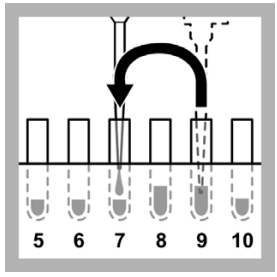


15. Do steps 13 and 14 againto put 1.5 mL of the sample into the test tube in position A10.

Figure 7 on page 89 shows the contents of the test tubes in Row A after this step is completed.



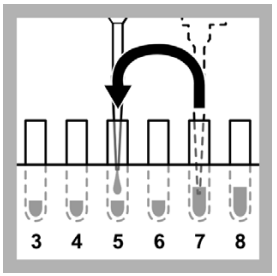
16. Pull the solution in A9 into the pipette 2 to 3 times to mix the sample dilution.



17. Start making the sample dilution series in Row A:

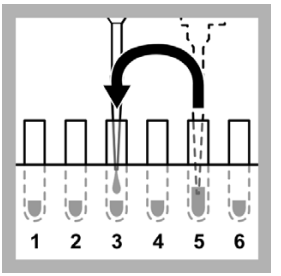
Pull in 1.5 mL of solution from A9 and put it into A7 using the pipette.

Pull the solution in A7 into the pipette 2 to 3 times to mix the sample dilution.



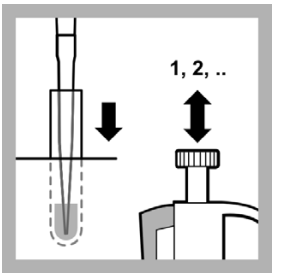
18. Pull in 1.5 mL of solution from A7 and put it into A5 using the pipette.

Pull the solution in A5 into the pipette 2 to 3 times to mix the sample dilution.

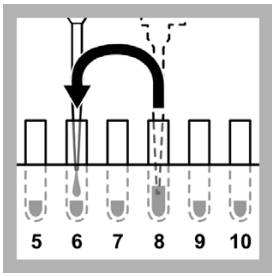


19. Pull in 1.5 mL of solution from A5 and put it into A3 using the pipette.

Pull the solution in A3 into the pipette 2 to 3 times to mix the sample dilution.

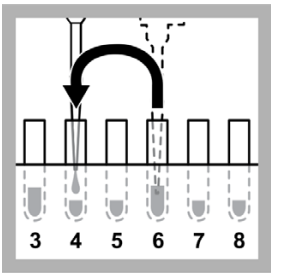


20. Pull the solution in A8 into the pipette 2 to 3 times to mix the sample dilution.



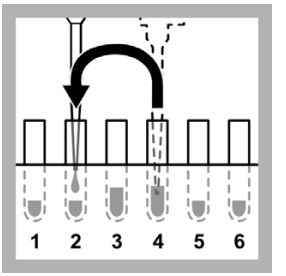
21. Pull in 1.5 mL of solution from A8 and put it into A6 using the pipette.

Pull the solution in A6 into the pipette 2 to 3 times to mix the sample dilution.



22. Pull in 1.5 mL of solution from A6 and put it into A4 using the pipette.

Pull the solution in A4 into the pipette 2 to 3 times to mix the sample dilution.



23. Pull in 1.5 mL of solution from A4 and put it into A2 using the pipette.

Pull the solution in A2 into the pipette 2 to 3 times to mix the sample dilution.

24. Keep the dilution series at 15 °C for at least 5 minutes to correct the temperature.

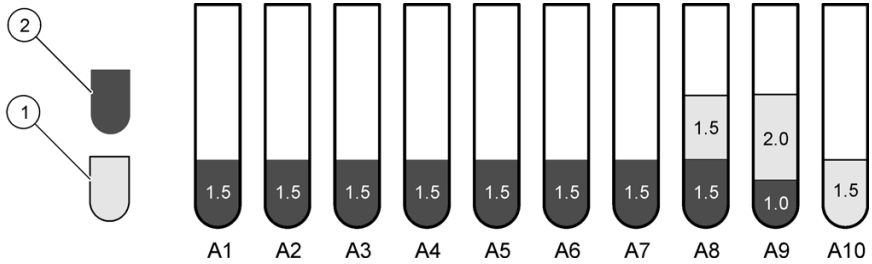


Figure 7 Before the dilution series is started - 9 dilutions, D 2 and higher

1 Sample	**2** 2% NaCl solution

11.10.2 Prepare a 3 dilution series (D 2 values and higher)

To make a 3 dilution series according to ISO 11348 of D 2 sample values and higher, do this procedure.

This procedure makes dilutions ranging from undiluted to a dilution ratio of 1:2 in row A. This corresponds to D values of 2 to 4 (Figure 8) in the test, as 0.5 mL of the sample dilution is added to 0.5 mL of the test suspension during the test in Row B and C. Adding test suspension to the sample dilutions increases the sample dilutions in row A by a factor of two as final test concentration.

Figure 8 shows the contents of the test tubes in Row A at the end of this procedure

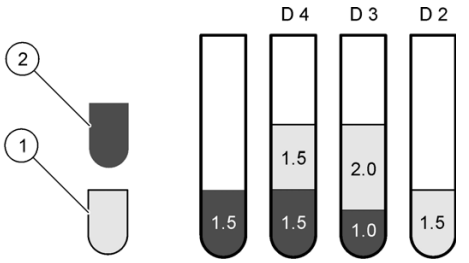
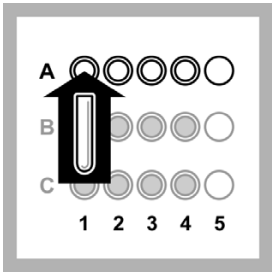
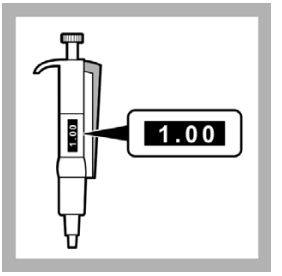


Figure 8 Dilution series - 3 dilutions, D 2 values and higher

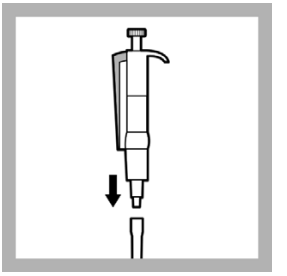
1 Sample	**2** 2% NaCl solution



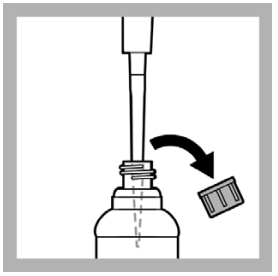
1. Put 4 empty test tubes into Row A of the LUMIStherm.



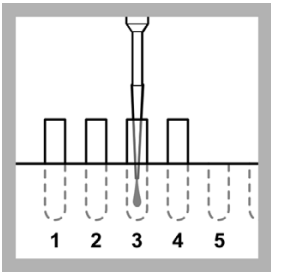
2. Set the 1.0 - 5.0 mL pipette to 1.0 mL.



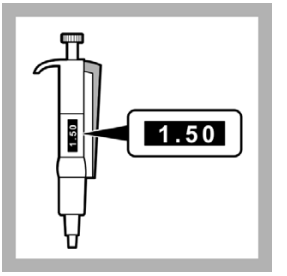
3. Put the end of the pipette into a clean pipette tip.



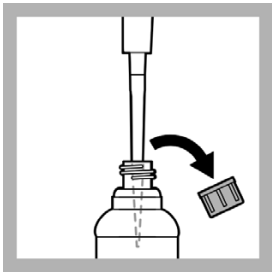
4. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 1.0 mL.



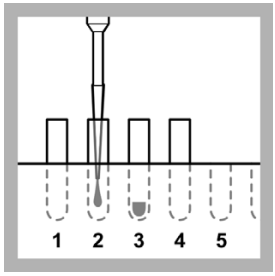
5. Slowly dispense the 2% NaCl solution into the test tube in position A3.



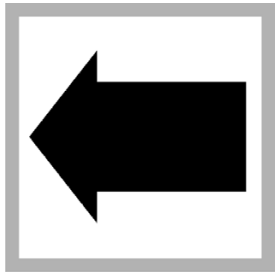
6. Set the 1.0 - 5.0 mL pipette to 1.5 mL.



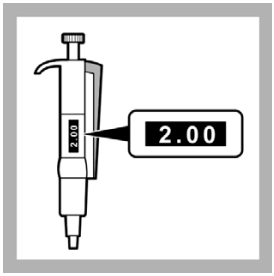
7. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 1.5 mL.



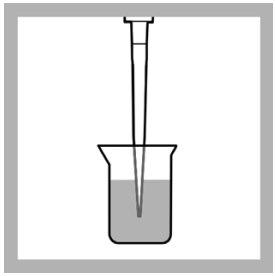
8. Slowly dispense the 2% NaCl solution into the test tube in position A2.



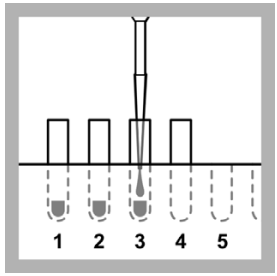
9. Do steps 7 and 8 againto put 1.5 mL of 2% NaCl solution into the test tube in position A1.



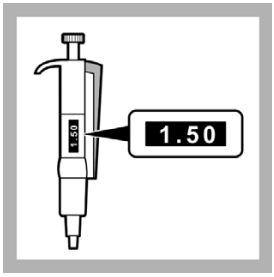
10. Set the 1.0 - 5.0 mL pipette to 2.0 mL.



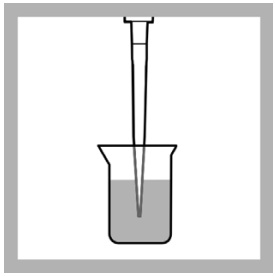
11. Put the tip of the pipette into the sample and slowly pull in 2.0 mL.



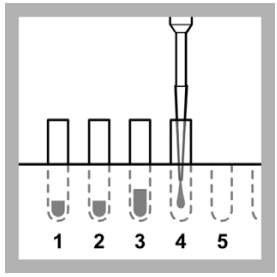
12. Slowly dispense the sample into the test tube in position A3.



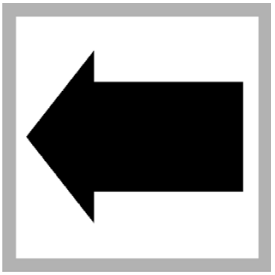
13. Set the 1.0 - 5.0 mL pipette to 1.5 mL.



14. Put the tip of the pipette into the sample and slowly pull in 1.5 mL.



15. Slowly dispense the sample into the test tube in position A4.



16. Do steps 14 and 15 againto put 1.5 mL of the sample into the test tube in position A2.

17. Keep the dilution series at 15 °C for at least 5 minutes to correct the temperature.

11.10.3 Prepare a 9 dilution series (D 1 values and higher)

To make a 9 dilution series according to ISO 11348 of D 1 sample values and higher, do this procedure.

This procedure makes dilutions ranging from undiluted to a dilution ratio of 1:8 in Row A. This corresponds to D values of 1 to 16 (Figure 9) in the test, as 0.5 mL of the sample dilution is added to 0.5 mL of the test suspension during the test in Row B and C. Adding test suspension to the sample dilutions increases the sample dilutions in row A by a factor of two as final test concentration.

Note: *The test tubes in the higher Row A positions are more concentrated. The pipette is moved from A9 to A4 (higher concentration to lower concentration) when making the dilution series, so the pipette tip does not need to be replaced during this procedure.*

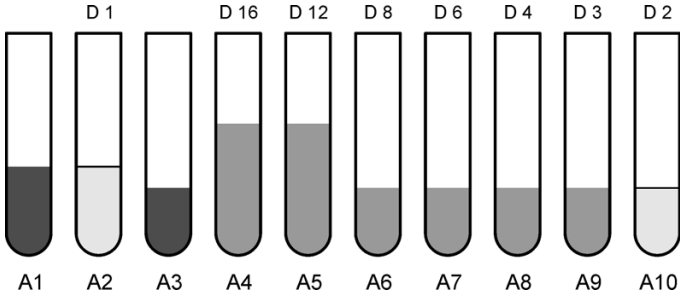
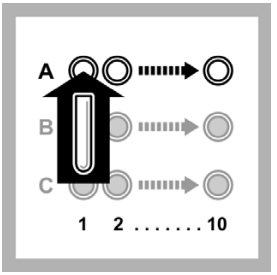
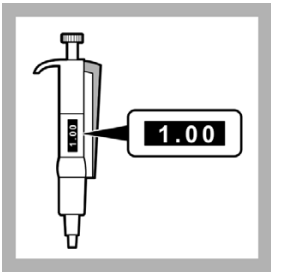


Figure 9 Dilution series - 9 dilutions, D 1 values and higher

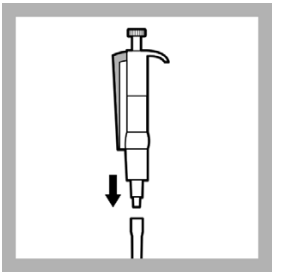
Tube	Contents
A1	2% NaCl (2.0 mL)
A2	Sample (2.0 mL)
A3	2% NaCl (1.5 mL)
A4, A5	2% NaCl and sample (3.0 mL)
A6 - A9	2% NaCl and sample (1.5 mL)
A10	Sample (1.5 mL)



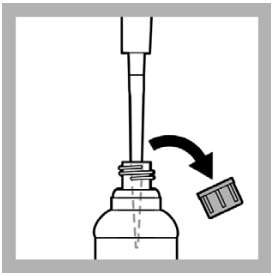
1. Put 10 empty test tubes into Row A of the LUMIStherm.



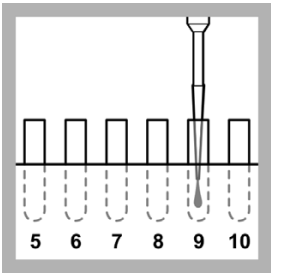
2. Set the 1.0 - 5.0 mL pipette to 1.0 mL.



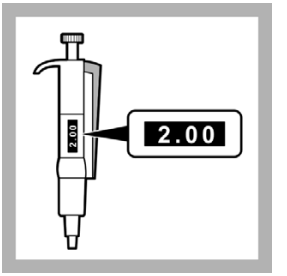
3. Put the end of the pipette into a clean pipette tip.



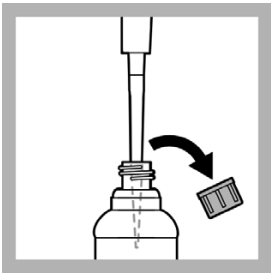
4. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 1.0 mL.



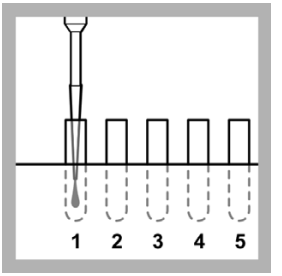
5. Slowly dispense the 2% NaCl solution into the test tube in position A9.



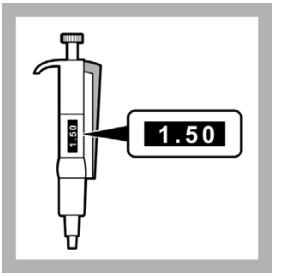
6. Set the 1.0 - 5.0 mL pipette to 2.0 mL.



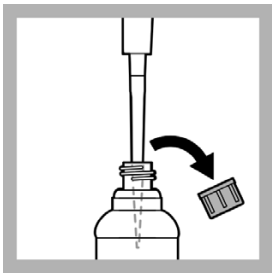
7. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 2.0 mL.



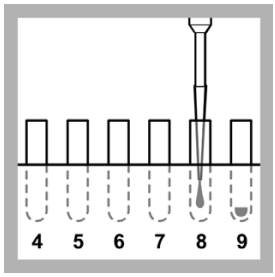
8. Slowly dispense the 2% NaCl solution into the test tube in position A1.



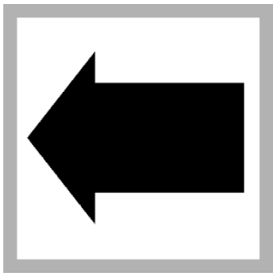
9. Set the 1.0 - 5.0 mL pipette to 1.5 mL.



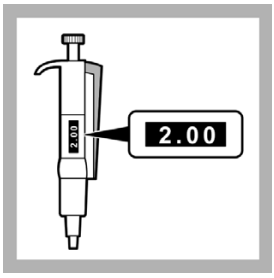
10. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 1.5 mL.



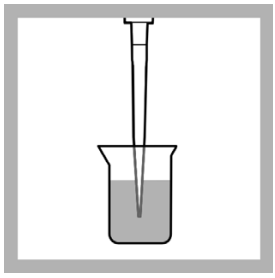
11. Slowly dispense the 2% NaCl solution into the test tube in position A8.



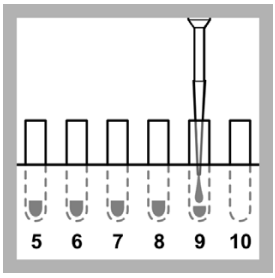
12. Do steps 10 and 11 againto put 1.5 mL of 2% NaCl solution in each test tube in positions A7, A6, A5, A4 and A3 .



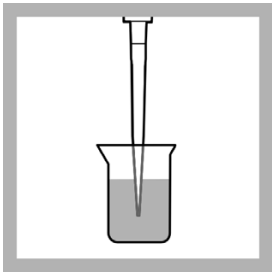
13. Set the 1.0 - 5.0 mL pipette to 2.0 mL.



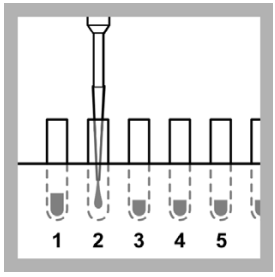
14. Put the tip of the pipette into the sample and slowly pull in 2.0 mL.



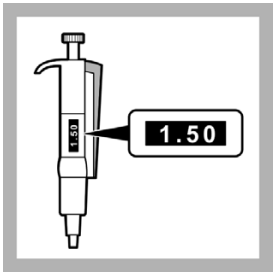
15. Slowly dispense the sample into the test tube in position A9.



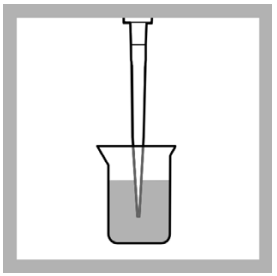
16. Put the tip of the pipette into the sample and slowly pull in 2.0 mL.



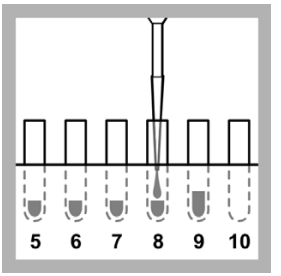
17. Slowly dispense the sample into the test tube in position A2.



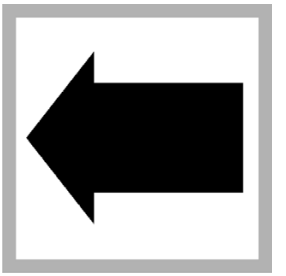
18. Set the 1.0 - 5.0 mL pipette to 1.5 mL.



19. Put the tip of the pipette into the sample and slowly pull in 1.5 mL.

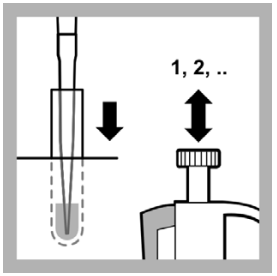


20. Slowly dispense the sample into the test tube in position A8.

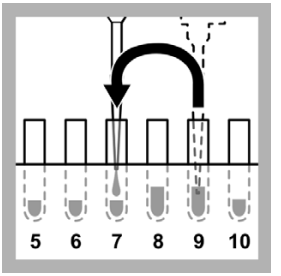


21. Do steps 19 and 20 againto put 1.5 mL of the sample into the test tube in position A10.

Figure 10 on page 97 shows the contents of the test tubes in Row A after this step is completed.



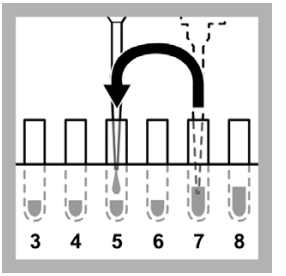
22. Pull the solution in A9 into the pipette 2 to 3 times to mix the sample dilution.



23. Start making the sample dilution series in Row A:

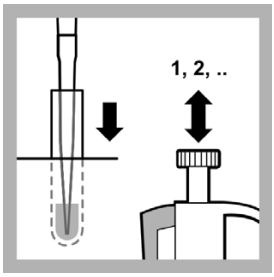
Pull in 1.5 mL of solution from A9 and put it into A7 using the pipette.

Pull in the solution in A7 into the pipette 2 to 3 times to mix the sample dilution.

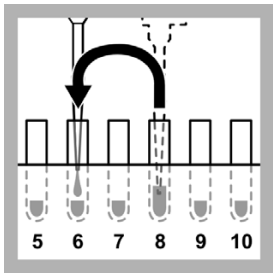


24. Pull in 1.5 mL of solution from A7 and put it into A5 using the pipette.

Pull in the solution in A5 into the pipette 2 to 3 times to mix the sample dilution.

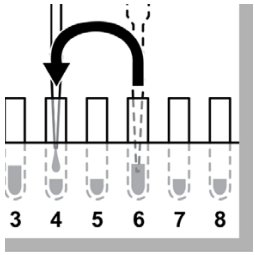


25. Pull the solution in A8 into the pipette 2 to 3 times to mix the sample dilution.



26. Pull in 1.5 mL of solution from A8 and put it into A6 using the pipette.

Pull in the solution in A6 into the pipette 2 to 3 times to mix the sample dilution.



27. Pull in 1.5 mL of solution from A6 and put it into A4 using the pipette.

Pull in the solution in A4 into the pipette 2 to 3 times to mix the sample dilution.

28. Keep the dilution series at 15 °C for at least 5 minutes to correct the temperature.

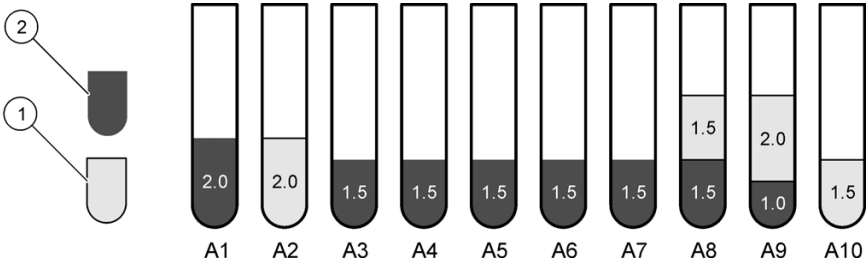


Figure 10 Before the dilution series is started - 9 dilutions, D 1 and higher

1 Sample	**2** 2% NaCl solution

11.10.4 Prepare a 3 dilutions series (D 1 values and higher)

To make a 3 dilution series according to ISO 11348 of D 1 sample values and higher, do this procedure.

This procedure makes dilutions ranging from undiluted to a dilution ratio of 1:1.5 in Row A. This corresponds to D values of 2 to 3 (Figure 11) in the test, as 0.5 mL of the sample dilution is added to 0.5 mL of the test suspension during the test in Row B and C. Adding test suspension to the sample dilutions increases the sample dilutions in row A by a factor of two as final test concentration.

Figure 11 shows the contents of the test tubes in Row A at the end of this procedure

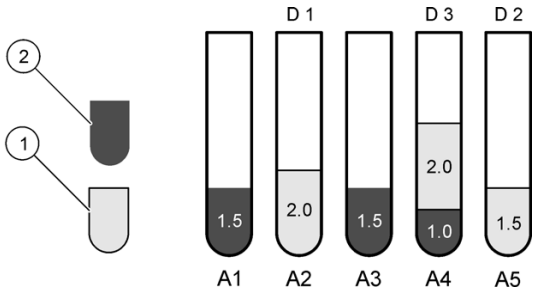
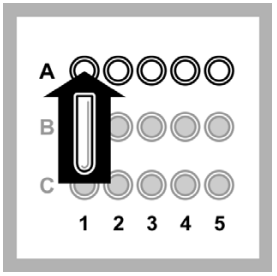
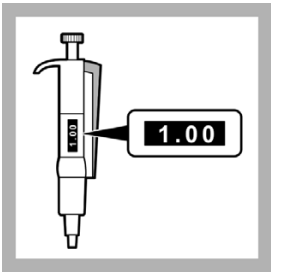


Figure 11 Dilution series - 3 dilutions, D 1 values and higher

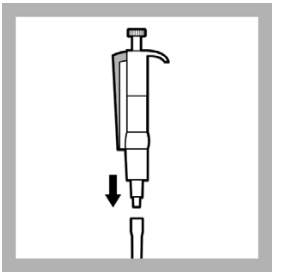
1 Sample	**2** 2% NaCl solution



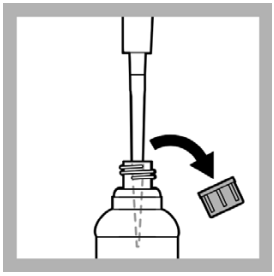
1. Put 5 empty test tubes into Row A of the LUMIStherm.



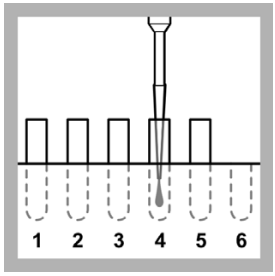
2. Set the 1.0 - 5.0 mL pipette to 1.0 mL.



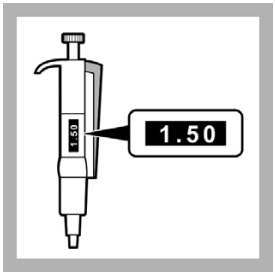
3. Put the end of the pipette into a clean pipette tip.



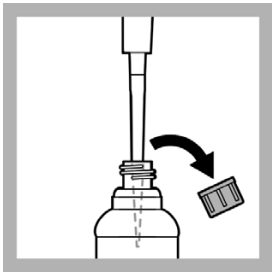
4. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 1.0 mL.



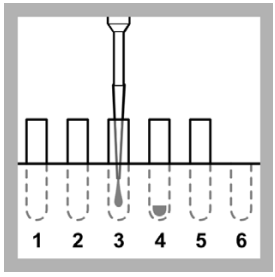
5. Slowly dispense the 2% NaCl solution into the test tube in position A4.



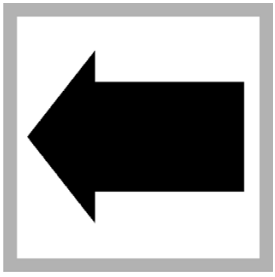
6. Set the 1.0 - 5.0 mL pipette to 1.5 mL.



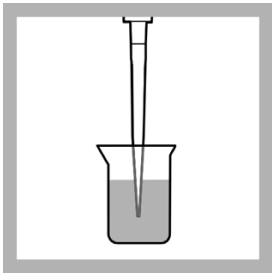
7. Put the tip of the pipette into the 2% NaCl solution and slowly pull in 1.5 mL.



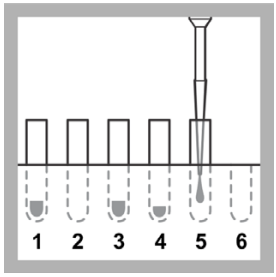
8. Slowly dispense the 2% NaCl solution into the test tube in position A3.



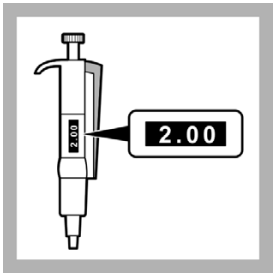
9. Do steps 7 and 8 againto put 1.5 mL of 2% NaCl solution into the test tube in position A1.



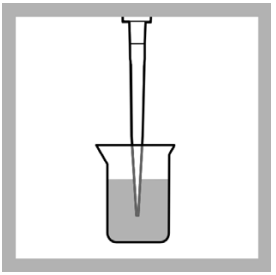
10. Put the tip of the pipette into the sample and slowly pull in 1.5 mL.



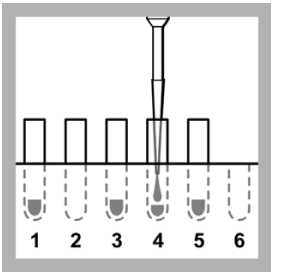
11. Slowly dispense the sample into the test tube in position A5.



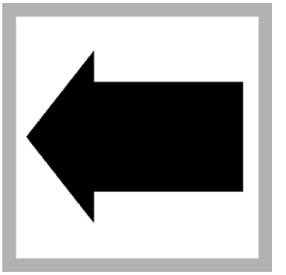
12. Set the 1.0 - 5.0 mL pipette to 2.0 mL.



13. Put the tip of the pipette into the sample and slowly pull in 2.0 mL.



14. Slowly dispense the sample into the test tube in position A4.



15. Do steps 13 and 14 againto put 2.0 mL of sample into the test tube in position A2.

16. Keep the dilution series at 15 °C for at least 5 minutes to correct the temperature.

11.11 Measure the light intensity of the test suspension

Measure the light intensity of the test suspension (luminescent bacteria) according to ISO 11348 using this procedure.

The Luminescent Bacteria Toxicity Test is a biological test method and the result is therefore strongly temperature-dependent. ISO 11348 states that the test must be done under temperature controlled conditions at 15 °C using a thermostat (LUMIStherm, LTV053).

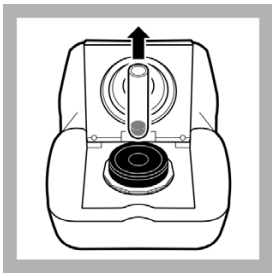
In some instances, if reconstitution is done at the optimum temperature and the test is carried out at 20 °C, the initial light made by the bacteria can be more than 1000 Eclox light units. This causes the error Detector Overload. If an error occurs, change the amplification settings from 0–1000 to 0–2000 light units and do the readings again (refer to Set the measurement range on page 18).

To measure the light intensity of the test suspension:

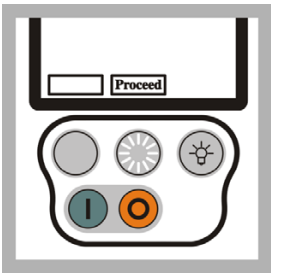
1. Push **ON** (green button) for several seconds to apply power to the luminometer.

   When the built-in tests are done, push **PROCEED**. The Main Menu is shown.
2. Select Luminescent Bacteria Test and push **ENTER**.
3. Select Measure and push **ENTER**.
4. Select Measure Luminescence and push **ENTER**.
5. Select one option that is shown:

   - To measure luminescence and save the results on the luminometer, select Measure Luminescence and Save and push **ENTER**.
   - To measure luminescence and manually record the measuring values on paper, select Measure Luminescence without saving and push **ENTER**.
   - To measure the luminescence and send the results to a PC, start LUMISsoft on the computer, start the test on LUMISsoft, and when Please select LSoft at the Luminometer is shown, select Measure Luminescence and Send to PC and push **ENTER**. The luminometer must be connected to a computer (refer to Connect the luminometer to a computer on page 23).
   - To measure the luminescence and print the results on a printer, select Measure Luminescence and Send to Printer and push **ENTER**. The luminometer must be connected to a printer (refer to Connect the luminometer to a printer on page 21).

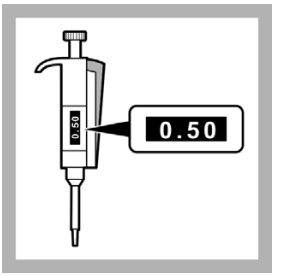


1. Open the luminometer lid and remove any sample that is in the cell. Close the lid.



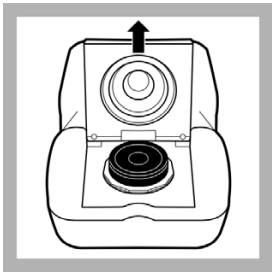
2. Push **PROCEED** to show the test status.

When the cell tests are done, push **PROCEED** again.



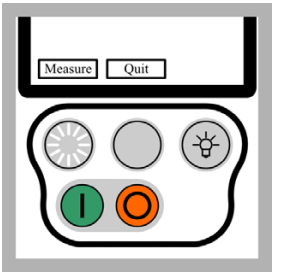
3. If testing D 2 values and higher, set the 0.2 - 1.0 mL pipette to 0.5 mL.

If testing D 1 values and higher, set the 0.2 - 1.0 mL pipette to 0.8 mL.

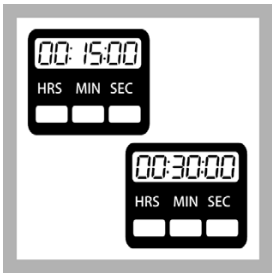


4. Open the luminometer lid.

5. Put the test tube in position B1 into the black test tube holder in the luminometer cell.



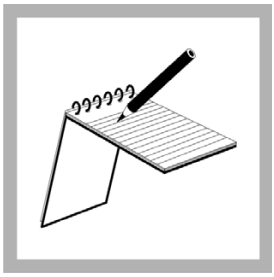
6. Close the luminometer lid. Push **MEASURE**.



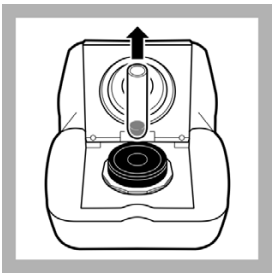
7. While B1 is being measured, set the timer to the correct contact time (e.g., 15 or 30 minutes).

Start the timer.

When the measurement is complete (approximately 15 seconds), the luminometer shows the relative light intensity of the test tube.



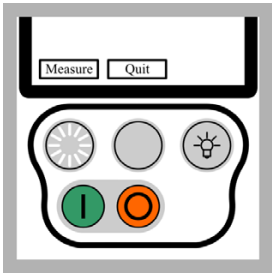
8. Record the measured value on a sheet of paper if the measured value is shown but not saved to the luminometer, saved to the computer or printed.



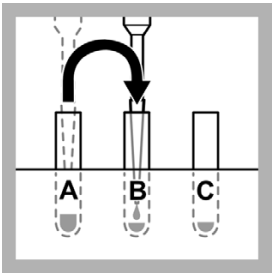
9. Open the lid of the luminometer.

Remove the test tube from the luminometer and put it back in the LUMISTherm.

10. Put the test tube in position C1 into the black test tube holder in the luminometer cell.



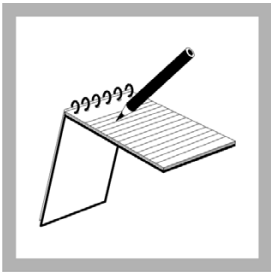
11. Close the luminometer lid. Push **MEASURE**.



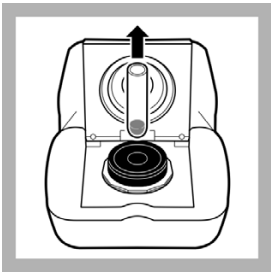
12. If testing D 2 values and higher, put the pipette in test tube A1 and pull in 0.5 mL of sample dilution.

If testing D 1 values and higher, put the pipette in test tube A1 and pull in 0.8 mL of sample dilution.

Put the pipette into the test suspension and slowly dispense the sample in B1. Mix with the pipette.

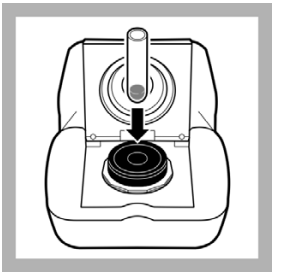


13. When the C1 measurement is done, record the measuring value on a sheet of paper if the measuring value is shown but not saved to the luminometer, saved to the computer or printed.

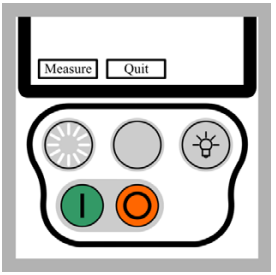


14. Open the lid of the luminometer.

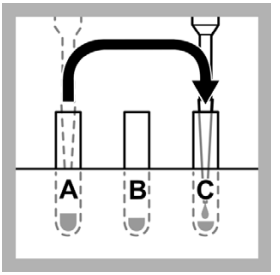
Remove the test tube from the luminometer and put it back in the LUMIStherm.



15. Put the test tube in position B2 into the black test tube holder in the luminometer cell.



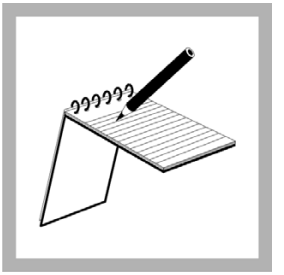
16. Close the luminometer lid. Push **MEASURE**.



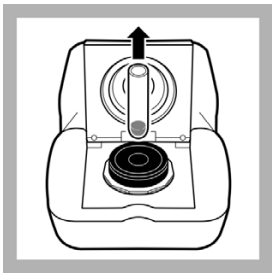
17. If testing D 2 values and higher, put the pipette in test tube A1 and pull in 0.5 mL of sample dilution.

If testing D 1 values and higher, put the pipette in test tube A1 and pull in 0.8 mL of sample dilution.

Put the pipette into the test suspension and slowly dispense the sample in C1. Mix with the pipette.

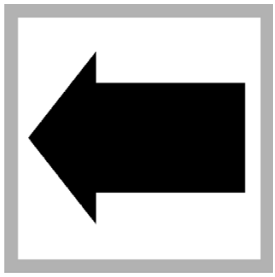


18. When the B2 measurement is done, record the measuring value on a sheet of paper if the measuring value is shown but not saved to the luminometer, saved to the computer or printed.



19. Open the lid of the luminometer.

Remove the test tube from the luminometer and put it back in the LUMIStherm.



20. Do steps 15 and 19 again until:

- All the test tubes in Row B and C have been measured and recorded moving from left to right (e.g., B2, C2, B3, C3, etc.).
- If testing D 1 values and higher, 0.8 mL of the sample dilution from the test tube in position A2 has been added to the test tubes in position B2 and C2.
- If testing D 2 values and higher, 0.5 mL of the sample dilution from the test tube in position A2 has been added to the test tubes in position B2 and C2.
- 0.5 mL of the sample dilution from each test tube in Row A (A3 and higher) has been added to the test tubes in Row B and C that have the same position number (e.g., from A**4** to B**4** and C**4**).

Note: *Add the sample dilution to each test tube in Row B and Row C immediately after the test tube is measured.*

11.12 Measure the light intensity of the test suspension after the sample dilutions are added

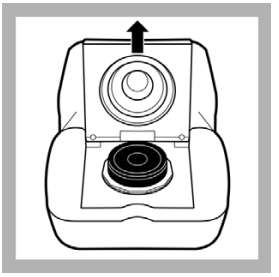
Measure the light intensity of the test suspension (luminescent bacteria) after the sample dilutions are added according to ISO 11348 using this procedure.

The Luminescent Bacteria Toxicity Test is a biological test method and the result is therefore strongly temperature-dependent. ISO 11348 states that the test must be done under temperature controlled conditions at 15 °C using a thermostat (LUMIStherm, LTV053).

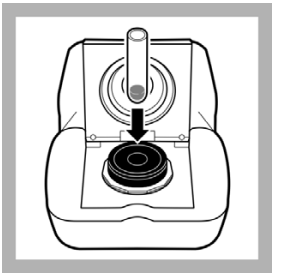
If the Luminescent Bacteria Toxicity Test is done at ambient temperature, record the temperature. The results of tests done at different temperatures cannot be compared directly.

To measure the light intensity of the test suspension after the sample dilutions are added:

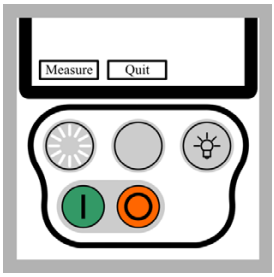
1. Wait until the contact time is completed.



2. Open the luminometer lid.

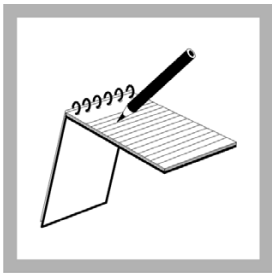


3. Put the test tube in position B1 into the black test tube holder in the luminometer cell.

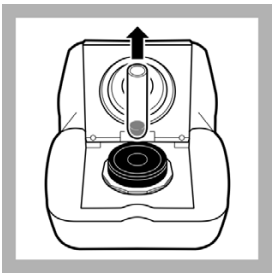


4. Close the luminometer lid. Push **MEASURE**.

When the measurement is complete (approximately 15 seconds), the luminometer shows the relative light intensity of the test tube.

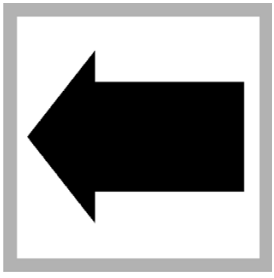


5. Record the measured value on a sheet of paper if the measured value is shown but not saved to the luminometer, saved to the computer or printed.



6. Open the lid of the luminometer.

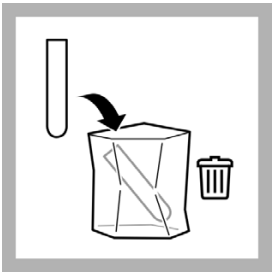
Remove the test tube from the luminometer and put it back into the LUMIStherm.



7. Do steps 3 to 6 again until all the test tubes in Row B and Row C have been measured.

Measure the test tubes in the same order that the light output of the test suspension was measured (e.g., B1, C1, B2, C2, etc.)

8. Put the solution in the test tubes into the waste bottle.

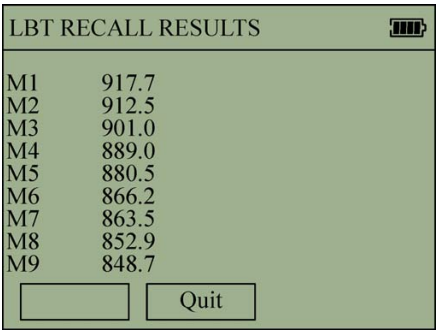


9. Put the test tubes into the waste bag.

11.13 Show or send previous results

Measured luminescent procedure values are stored by an indicator counter M1 to Mx (refer to Figure 12). The counter starts with M1 every time the storage is erased from the luminometer.

Figure 12 Example measurement luminescence results



To show previous results on the luminometer for the Luminescent Bacteria Test (LBT), do the procedure in this section.

To send previous results to a computer:

Note: *At this stage, the results cannot be sent to the LUMISsoft 4.*

1. Do the steps in Connect the luminometer to a computer on page 23.
2. Start LUMISsoft.
3. In LUMISsoft, select Transfer, Options, Interface Protocol, Connect.
4. Do the procedure in this section.

To send previous results to a printer, do the steps in Connect the luminometer to a printer on page 21 and then do the procedure in this section.

To show or send previous results saved on the luminometer:

1. Push **ON** (green button) to apply power to the luminometer.

   The luminometer does built-in tests that make sure that the electronics and software are operating correctly.
2. When the built-in tests are done, push **PROCEED**.

   The Main Menu is shown.
3. Select Luminescent Bacteria Test and push **ENTER**.

   The LBT Main Menu is shown.
4. Select Previous Results and push **ENTER**.

The Previous Results Menu is shown.

5. Select Show Previous Measurements and push **ENTER**.

   The Previous Measurements Menu is shown.

6. To show all or send all of the results saved on the luminometer, select one option:

   - To show the results on the luminometer, select Show all (R1 to Rx) and push **ENTER**.
   - To send the results to the computer, select Send all (R1 and Rx) to PC and push **ENTER**.
   - To send the results to the printer, select Send all (R1 to Rx) to Printer and push **ENTER**.

Note: *At this stage, the results cannot be sent to the LUMISsoft 4.*

7. To show or send a specific range of results saved on the luminometer, select one option:

   - To show the results on the luminometer, select Show selection and push **ENTER**.
   - To send the results to the computer, select Send selection to PC and push **ENTER**.
   - To send the results to the printer, select Send selection to Printer and push **ENTER**.

8. If an option in step 7 was selected, select the data to be recalled:

   a. Select the starting indicator in the From field. Push **SELECT** to change the value. Then push **PROCEED**.

   b. Select the ending indicator in the To field. Push **SELECT** to change the value. Then push **SHOW**.

9. Push **PROCEED** to show more results.

Section 12 Maintenance

Important Note: *All cleaning and maintenance of the Eclox™ Rapid Response Test Kit should be done in a suitable clean, dry area. Make sure that the kit is clean before removing any access or battery covers. Do not let foreign material enter the kits as equipment damage can occur.*

12.1 General maintenance

The Eclox Rapid Response Test Kit is made for field use. Routine maintenance does not have to be done if all cleaning, test and calibration procedures are done.

12.1.1 Cleaning the kit

Keep the kit in good condition and clean to get reliable results. Clean the kit before it is put into storage. Complete a decontamination form and put the form with the kit.

12.1.2 Cleaning the luminometer

Keep the luminometer clean at all times. If the surface is dirty, clean the surface with a damp cloth.

Important Note: *Do not let water get into the luminometer cell. If water gets into the cell, remove the cell insert and remove the moisture with a clean, dry cloth. Replace the cell insert.*

12.2 Decontamination

If the Eclox Rapid Response Test Kit comes into contact with any chemical warfare (CW) agent, decontaminate the kit before it is used again.

When the carrying case for the Eclox Rapid Response Test Kit is closed, the kit is waterproof and can be sprayed/wetted. The case is chemically made hard.

The outside of the luminometer is also CW agent resistant and can be decontaminated after a CW attack. None of the other kit components are CW agent resistant and in the event of exposure (when the lid is open), the kit is contaminated and must be quarantined for disposal.

12.3 Battery replacement

12.3.1 Luminometer battery replacement

1. Remove any excess water from the surface of the luminometer. Measurement errors will occur if water gets into the meter.

2. Remove the battery cover of the luminometer with the Battery Cover Screw Tool.

3. Remove the batteries from the luminometer and dispose of them in accordance with local operating procedures.

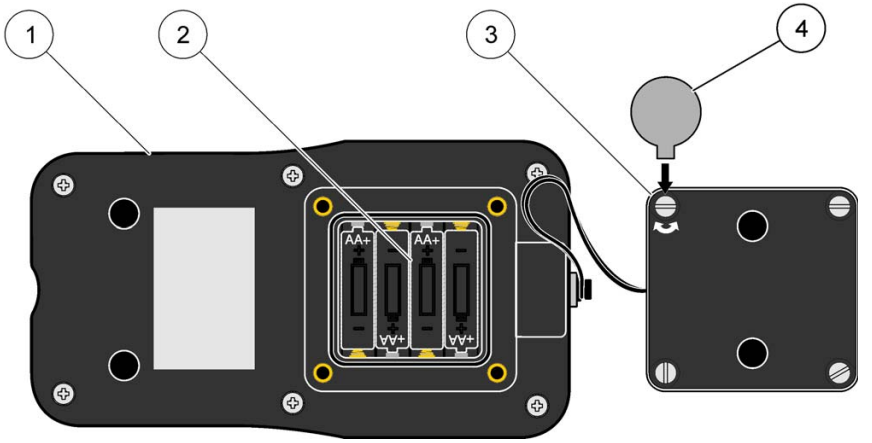
4. Put four new batteries (AA, Alkaline) in the luminometer. Make sure the battery polarity is correct.

5. Put the battery cover on the luminometer with the Battery Cover Screw Tool.

6. Push **ON** (green button) to apply power to the luminometer.

7. Do the pre-deployment checks (refer to section 3.2.1, Test the operation on page 16).

Figure 13 Replace the luminometer batteries



1.	Luminometer	**3.**	Battery compartment cover
2.	Battery compartment	**4.**	Battery Cover Screw Tool

Table 4 Luminometer troubleshooting

Display	Fault	Corrective action
—	Detector overload	Change the measurement range to the 0–2000 range (refer to section 3.2.3, Set the measurement range on page 18).
—	cannot read the display	Change batteries (refer to section 12.3.1, Luminometer battery replacement on page 112).
—	Chemiluminescence line develops in a bell shaped curve.	The chemiluminescence reagents are weak and need to be replaced.
—	Negative percent inhibition	Do the reference measurement again. Salt could be in the sample or the reagents may be faulty.
—	—	Change the contrast.
Error	Database is full. No new measurements can be saved.	Erase all measurements (refer to section 3.2.2, Erase the results saved on the luminometer on page 17).
01	System RAM test failed	Memory failure. Contact technical support.
02	System EPROM test failed	Memory failure. Contact technical support.
03	LCD display test failed	Continue operation. If fault occurs again, contact technical support.
04	Non-volatile RAM test failed	Memory failure. Contact technical support.
05	Storage measurements corrupt	All saved measurements will be erased. Continue operation. If fault occurs again, contact technical support.
06	Configuration settings corrupt	LCD contrast and measurement range will be reset. Continue operation. If fault error occurs again, contact technical support.
07	Usage counter corrupt	Usage counter will be reset to zero. Continue operation. If fault occurs again, contact technical support.
08	A/D input failure	Failed measurement. Contact technical support.
09	Reference LED failure	Failed measurement. Contact technical support.
11	Reference LED reading fault	Failed measurement. Contact technical support.
100	Cannot clear measurements	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.
101	Cannot store range settings	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.

Table 4 Luminometer troubleshooting

Display	Fault	Corrective action
102	Cannot store contrast settings	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.
103	An internal error has occurred	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.
104	Cannot read signal level	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.
105	Cannot recall measurements	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.
106	Cannot store usage counter	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.
107	Cannot store measurements	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.
200	Initialization fault	Remove power and then apply power to the luminometer. If fault occurs again, contact technical support.

Section 14 Replacement parts and accessories

Replacement parts

Description	Qty	Item number
Beaker, 50 mL, plastic	each	108041
Battery Cover Screw Tool	each	2888000
Color comparator box	each	173200
Color disc, 0–100 and 0–500 color units	each	9262500
Color viewing tube, glass	6/pkg	173006
Cuvette holder	each	2887900
Cuvette and 1000 uL pipette tip set	28/pkg	2887400
Disc program	each	2888100
Long path viewing adapter	each	2412201
Luminometer	each	2887000
Pesticide test clip	each	2887700
Pipet, liquid transfer, 1000 µL (Blue)	each	2887300
Pipet, liquid transfer, 100 µL (Yellow)	each	2887100
Pipette, liquid transfer, 100 uL, tip set	each	2887200
Serial comms. lead (cable) , luminometer	each	2888200
Test record sheets	15/pkg	2888300
Waste bottle, 250 mL	each	2888400
Chemiluminescence Toxicity Test		
Chemiluminescent Reagent Set, 100 tests	each	94-9004
Cuvette and 1000 µL Pipet Set	2	2887400
Cuvette and 1000 uL Pipette Tip Set	each	2887400
Cuvette for Eclox, 500 pack	each	30-0015
100 µL Pipet Tip Set	2	2887200
Chemiluminescence Test Kit for 50 tests (includes CT deionized water, CT Reagent 1, CT Reagent 2CT Reagent 2 Buffer, CT Reagent 3, CT Reaget 3 concentrate, CT pre-conditioner)	each	2887500
Eclox Replacement Reagent Set (includes 50 chemiluminescent reagent sets, 25 pesticide/ nerve agent tests, 50 free chlorine, 50 total chlorine, 100 Hach Arsenic Tests, 10 pH buffer (Singlets) 4.01 from Hach and 10 pH buffer (Singlets) 7.00 from Hach)	each	2886900

Replacement parts (continued)

Description	Qty	Item number
Arsenic Test		
Cap, Arsenic Test Kit	each	4934800
Cotton balls		257201
EZ Arsenic Reagent #1	each	2822999
EZ Arsenic Reagent #2	each	2823099
EZ Arsenic Reagent Set (Reagent #1 and #2)	each	2823200
Lead acetate, 100 mL	each	1458042
Reaction bottle, arsenic	each	2800200
Sample cell, 10mm	each	2627600
Test strips, dual RG	each	2800150
Chlorine Test		
Color comparator box	each	173200
Color disc, DPD chlorine, 0–3.4 mg/L	each	990200
Color viewing tube, plastic, with cap	4/pkg	4660004
DPD free chlorine reagent powder pillows	100/pkg	1407799
DPD total chlorine reagent powder pillows	100/pkg	1407699
Caps, plastic color viewing tubes (4660004)	4/pkg	4660014
Chlorine standard solution, 50–75 mg/L, 2-mL PourRite® ampule	20/pkg	1426820
Color viewing tube, glass	6/pkg	173006
Stoppers, glass color viewing tubes (173006)	6/pkg	173106
Pesticide Test		
Pesticide/ Nerve Agent Test coupons, 25 tests	each	2887600

Replacement parts (continued)

Description	Qty	Item number
pH and TDS		
Pocket Pro™+ Multi 2 Tester	each	9532800
pH/Cond/TDS/Salinity sensor, replacement	each	9532801
AAA alkaline batteries	4/pkg	4674300
SINGLET™, 4.01 and 7.00 pH	10 each	2769920
SINGLET™, 12.88 ms/cm	20/pkg	2771520
SINGLET™, 1413 µS/cm	20/pkg	2771420
SINGLET™, 147 µS/cm	20/pkg	2771320
Luminescent Bacterial Test		
Luminescent bacteria reagent, freeze-dried (vials for 50 ml reagent solution)	12/pkg	LCK491
Luminescent Bacteria Accessories Kit (includes case, reconstitution solution, dilution solution, 2% NaCl solution, NaCl solid in a bottle with a dosing spoon, plastic test tubes for Eclox, reaction vessels, rack for 8 reaction vessels, stand for 40 test tubes, variable pipette 0.2-1.0 mL, variable pipette 1.0-2.0 mL, pipette tips and timer clock)	each	LCW490
Case	each	4660800
Dilution solution, 1000 mL	each	LCX048
2% NaCl solution, 250 mL	each	LCK481
NaCl solid in bottle with dosing spoon, 25 g	each	LCX058
Pipette, variable, 0.2 - 1.0 mL	each	BBP078
Pipette, variable, 1.0 - 5.0 mL	each	BBP065
Pipette tips for variable pipette BBP078	100/pkg	BBP079
Pipette tips for variable pipette BBP065	75/pkg	BBP068
Plastic test tubes for Eclox	500/pkg	LZP1480
Rack for 8 reaction vessels	each	LYW918
Reaction vessels with cap	5/pkg	LZP065
Reconstitution solution, 50 mL	each	LCX047
Stand for 40 test tubes	each	ETS018
Timer clock	each	LZC902

Appendix A Luminescent bacteria risks

This appendix contains risk assessment information for Photobacterium fisheri (synonym: Vibrio fischeri) manufactured by HACH-LANGE GmbH in Germany.

The Luminescent Bacteria Toxicity Test reagent contains freeze-dried or liquid-dried Photobacterium fisheri bacteria. Photobacterium fisheri luminescent bacteria are well known as non-pathogenic and innocuous.

The origin of Photobacterium fisheri is the strain number DSM 7151. The bacteria are multiplied but not changed.

The bacteria are used as indicator organisms to identify the toxicity of environmental or chemical samples. The Luminescent Bacteria Toxicity Test procedure has been standardized by ISO (International Standard Organisation) ISO 11348-1, -2, -3.

A.1 Risk specifications

Table 5 gives the risk specifications for Photobacterium fisheri.

Table 5 Risk specifications

Strain number	DSM 7151 - Vibrio fischeri (Beijerinck 1889) Lehmann and Neumann 1896AL (Bacteria) © by DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany Name Vibrio fischeri (Beijerinck 1889) Lehmann and Neumann 1896AL DSMZ number 7151 = NRRL-B-11177 = ATCC 49387
Restrictions Risk Group 1 (harmless bacteria)	ATCC Number: 49387 NRRL Number: B-11177 Organism: Photobacterium phosphoreum (Cohn) Beijerinck Designations: NRRL B-11177 Depositors: NRRL
Biosafety Level	Biosafety Level 1 ATCC: American Type Culture Collection; NRRL: ARS Culture Collection, Northern Regional Research Laboratory

A.2 Biosafety Level 1 information[1]

Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and

[1]From *Biosafety in Microbiological and Biomedical Laboratories*, (BMBL) 4th Edition (HHS Publication number (CDC) 93-8395. U.S. Department of Health and Human Services, Centres for Disease Control and Prevention and National Institutes of Health; U.S. Government Printing Office: Washington DC; 1999.

characterized strains of viable microorganisms that are not known to consistently cause disease in healthy adult humans. Bacillus subtilis, Naegleria gruberi, infectious canine hepatitis virus, and exempt organisms under the NIH Recombinant DNA Guidelines are representative of microorganisms meeting these criteria.

Many agents that are not normally associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand cleaning.

A.3 Disposal

The luminescent bacteria are harmless and can be put down the laboratory drain. Make sure to dispose of toxic samples correctly. Contact the local regulatory agency for correct disposal information.

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