



---

# **TOX-SCREEN<sup>3</sup> Test Kit**

## **User Guide**

### **General Limited Warranty**

CheckLight warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. CheckLight makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

Copyright 2009 CheckLight Ltd

## Table of contents

page

1- Introduction.....	6
About This Manual .....	6
What Is the TOX-SCREEN <sup>3</sup> Assay? .....	6
How Does the Kit Work? .....	7
What is Included in the Kit .....	7
Estimating the concentration of toxicants in the tested water sample ....	8
2- Performing the Assays.....	9
General Outline of the Assay Procedures .....	9
Important Factors That May Affect an Assay .....	9
Cleanliness.....	9
Accuracy .....	10
Reagent Freshness and Storage .....	11
Negative Control .....	11
Preparations .....	11
Buffers .....	11
Positive Controls .....	11
Dechlorination of Water Samples .....	12
Biosensor .....	12
Preparing the Biosensor .....	12

3- Specific Assay Procedures.....	13
Determination of IC50 - Protocol 1 .....	13
Equipment and Reagents Required .....	13
Solutions to Prepare Before Beginning the Assay .....	14
Water Sample.....	14
Assay Buffer .....	14
Negative Controls .....	14
Positive Control .....	14
Test Procedure.....	14
Dilute the Water Sample .....	14
Add Biosensor Incubate and Measure .....	15
On-Site Testing - Protocol 2 .....	17
Equipment and Reagents Required .....	17
Solutions to Prepare Before Beginning the Assay .....	17
Negative Controls .....	17
Positive Controls .....	18
Test Samples .....	18
Test Procedure .....	18
Rapid Pre-Screening - Protocol 3 .....	20
Equipment and Reagents Required .....	20
Solutions to Prepare Before Beginning the Assay .....	20

Positive Control .....	20
Negative Control .....	21
Test Procedure .....	22
4 Troubleshooting .....	23
5 Examples of the Use of the Kit .....	24
Using The Kit as Part of an Early Warning System for Determining Dangerous Changes in Drinking Water Quality .....	24
Introduction .....	24
Step 1 .....	24
Step 2 .....	24
Step 3 .....	24
Example of Screening Results .....	26
Using the Kit for Effluent Toxicity Monitoring .....	28
Introduction .....	28
Procedure .....	28
Step 1 – what dilution of sample exhibits 20% inhibition in luminescence?	30
Step 2 – what is the threshold level of the sample? .....	31
Step 3 – routine monitoring for changes in toxicity level .....	32
6 Frequently Asked Questions .....	33

# Introduction

## About This Manual

This manual contains the instructions for how to use the TOX-SCREEN<sup>3</sup> Test Kit. The kit is easy to use and provides quick accurate results as long as the instructions are followed. It is therefore very important that you read and understand all of Chapter 2 **before** starting the assay.

If you encounter any problems while performing the assay read over Important **Factors That May Affect an Assay** on page 9, and find a list of problems that you may encounter in **Specific Assay Procedures**, page 13.

## What Is the TOX-SCREEN<sup>3</sup> Assay?

The kit is for screening water from sources such as

- Ground water
- Finished drinking water
- Surface water
- Runoff water

A list of all the compounds that the kit is capable of detecting is constantly being updated as more chemicals are tested; it can be provided upon request.

The TOX-SCREEN<sup>3</sup> Test Kit should be used as part of an early warning system for water contamination testing. Its purpose is not to replace comprehensive chemical analysis testing. It should be regarded as a general qualitative test that provides rapid indication of dangerous changes in water quality.

## How Does the Kit Work?

The TOX-SCREEN<sup>3</sup> test is based on using light-producing (luminous) bacteria as very sensitive and accurate biosensors. The idea behind the assay is simple; toxic compounds in water cause the luminous bacteria to change the level of emitted light (in most cases, by inhibition).

The test kit includes Assay Buffers, freeze-dried luminous bacteria, positive control solutions, and plastic tubes. A luminometer is required to read the results.

The testing procedure is simple as well: a few steps that can be performed either in the lab or on site and can be used by water companies, health and environmental supervising authorities.

For more detailed information about how the kit works see **Frequently Asked Questions**, page 33.

## What is Included in the Kit

The following materials are provided with the kit. Make sure that all are present before proceeding with the assay:

- Biosensor - stoppered vials holding freeze-dried luminous bacteria
- Storage Buffer
- Pro-Metal concentrated Assay Buffer
- Pro-Organic concentrated Assay Buffer
- Positive control concentrated solutions: copper chloride and sodium chloroacetate
- Empty test tubes
- Luminometer

## Estimating the concentration of toxicants in the tested water sample



**We recommend that you use the provided Excel module for rapid and simple data analysis.**

The quantitative determination of water toxicity is **Protocol 1** - carried out by running a set of double dilutions of the water in question in the provided Assay Buffer followed by the addition of hydrated culture of luminous bacteria. The level of light obtained in all dilutions, as well as in the control samples, is recorded after 15 minutes incubation. See Determination of IC50, page 13.

The common endpoint for toxicity tests is expressed in relative values, termed EC50 (Effective Concentration of 50%), or IC50 (Inhibition Concentration of 50%) that is defined as the minimal concentration of tested water (in %) that results in 50% change of light output, as compared to light recorded in the clean reference water under defined assay conditions. Higher sensitivity can be obtained if one calculates EC30, EC20, or the Lowest Observable Effect Value (LOEC).

**Protocol 3** - Rapid Pre-Screening and **Protocol 2** - On-Site Testing describe sample testing at a single concentration (80%), hence, relative toxicity can not be determined. These protocols are ideal for rapid screening of multiple samples, and for testing low toxicity samples on-site or in the lab.

## 2 - Performing the Assays

### General Outline of the Assay Procedures

The assays are performed in the following steps:

1. Collect the sample
2. Prepare all reagents and equipment
3. Prepare tubes with Assay Buffers
4. Dilute (Depending on the assay)
5. Prepare positive control & negative control
6. Add water sample
7. Add Biosensor
8. Incubate
9. Read results
10. Interpret results

### Important Factors That May Affect an Assay

The accuracy of the results can be affected by a number of factors. It is very important to keep these factors in mind while performing the assay.

#### Cleanliness

Due to the high sensitivity of the assay, care should be taken to keep all tubes, plastic tips, and pipettes extremely clean. Keep in mind the following Do and Don't list:

### Do:

- Wash the plastic tips several times with clean water before use
- Work in a clean manner to keep the reagents from getting contaminated
- Since most errors are due to operator mishandling, it is advisable to run samples in duplicates to make sure test is performed accurately (+/- 15-20%)
- If test results are positive (i.e.,  $\geq 50\%$  light inhibition) – repeat test procedure to ensure reliability of results
- Keep incubation and measurement temperature constant

### Do not:

- Do not reuse the test tubes
- Do not wash pipettors, pipette tips or glassware with detergent, acids or solvents. Use water only

### Accuracy

Due to the great sensitivity of the kit it is very important to add the reagents in exactly the right amounts. Therefore:

- Make sure you use a repeat dispenser (automatic pipettor) with 0.5ml syringe to dispense 10 microliter of hydrated bacteria.
- Make sure that the pipette tip is firmly attached to the pipettor each time you add a reagent.
- Before pipetting double-check that the pipettor is set to the correct amount.
- Check that there are no air bubbles inside the pipette tip.

## Reagent Freshness and Storage

Make sure that all reagents are stored under appropriate conditions both in storage and after preparation.

## Negative Control

Each assay requires a sample that we are sure contains either no or sub-toxic amounts of the compounds that we are screening for. If indicated this sample must come from local water as the exact composition of water varies from place to place. For this same reason distilled or de-ionized water should not be used except where indicated.

## Preparations

Before beginning an assay prepare the following:

### Buffers

Two concentrated (x5) Assay Buffers are provided - "Pro-Metal" to test for the presence of cationic heavy metals & metalloids, and "Pro-Organic" to test for the presence of toxic organic compounds. It is recommended to take two aliquots of the sampled water and test with both buffers in parallel. Once diluted in water, make sure the pH of the Pro-Organic Buffer is 4.5, and the pH of the Pro-Metal Buffer is 7.5.

Also provided is Storage Buffer for hydration and storage of the suspended bacteria. The shelf life of all buffers is 12 months when stored at 4°C.

### Positive Controls

Concentrated (x100) stock solutions of sodium chloroacetate and copper chloride are provided as positive controls. It is recommended to use them as positive controls in each test run to confirm proper performance of the test protocol. When testing these agents at 1:100 dilution in clean reference water a light inhibition of at least 50% should be noted; if not, the test was not performed properly – re-read protocol carefully, and repeat experiment.

## Dechlorination of Water Samples

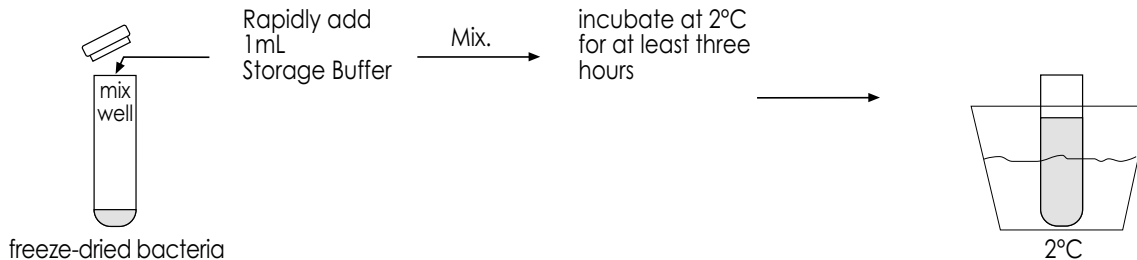
The presence of chlorine and its by-products leads to rapid decay of bacterial bioluminescence. When required, up to 2 ppm chlorine can be chelated by addition of sodium thiosulphate (2 ppm). Higher concentrations of chlorine should be properly diluted in clean water prior to addition of thiosulphate, or removed by other means. Keep in mind, though, that thiosulfate might negate the activity of some toxicants, such as, cyanide, mercury and lead, at certain concentrations.

## Biosensor

A freeze-dried preparation of the luminescent marine bacterium *P. leiognathi* SB. The shelf life of this reagent is one year when stored in a deep-freezer (-10° – -20° C). Do not store the Biosensor in a self-defrosting freezer because it defrosts by warming up periodically.

## Preparing the Biosensor

Once reconstituted with Storage Buffer, the Biosensor should be quickly and carefully mixed well. The Biosensor suspension may be used immediately, however, for optimal and reliable results it is essential to incubate for **at least three hours** at 2°-4°C before use. To minimize temperature changes when removing tube for testing it is recommended to store the tube in the provided water-filled tube. Avoid unnecessary exposure to ambient temperature. Under these conditions aliquots of the Biosensor may be drawn repeatedly for up to 5 days.



## 3 Specific Assay Procedures

### Protocol 1- Determination of IC50

The degree of water toxicity can be expressed as IC50. Where IC50 is defined as the concentration of the tested water (in %) that results in 50% inhibition of the light level obtained in the clean water control. The provided Excel module assists you in automatically calculating this value from the generated data.

### Equipment and Reagents Required

- Two sets of 9 tubes
- 1.8 mL Pro-Metal and Pro-Organic concentrated Assay Buffers, each.
- A tube with 0.8mL double distilled or HPLC grade water + copper (dilute stock 1:100)
- A tube with 0.8 mL double distilled or HPLC grade water + sodium chloroacetate (dilute stock 1:100)
- 180µl of hydrated Biosensor maintained in Storage Buffer (from stock kept at 2°-4°C)
- Pipettors (repeat dispenser & regular) and tips
- Heating plate & water bath (alternatively, temperature controlled air incubator)
- Optional – Vortex for mixing samples
- Luminometer
- Double distilled or HPLC grade water to serve as negative control

## Solutions to Prepare Before Beginning the Assay

### Water Sample

1. Put 1.6 ml of the sample water to be tested in to test tube #1
2. Add 0.4 ml of concentrated buffer, either Pro-Metal or Pro-Organic depending on the suspected pollutant

### Assay Buffer

3. Dilute the concentrated assay buffer 1:5 in double distilled or HPLC grade water. In this example add 2 ml concentrated assay buffer to 8 ml of clean water. Mix well. The exact volume will depend on how many tubes there are, in this example we are using 9 tubes
4. Dispense 1 ml into each of the remaining 8 tubes. Mark the tubes #2 -#9

### Negative Control

Put tubes #7 and #8 (that contain only diluted buffer in clean water) aside to be used as negative controls

### Positive Controls

To tube #9 add 10  $\mu$ l of copper stock solution - if using Pro-Metal Buffer; or 10 $\mu$ L of sodium chloroacetate stock solution -if using Pro-Organic Buffer. Mix well and put aside

## Procedure

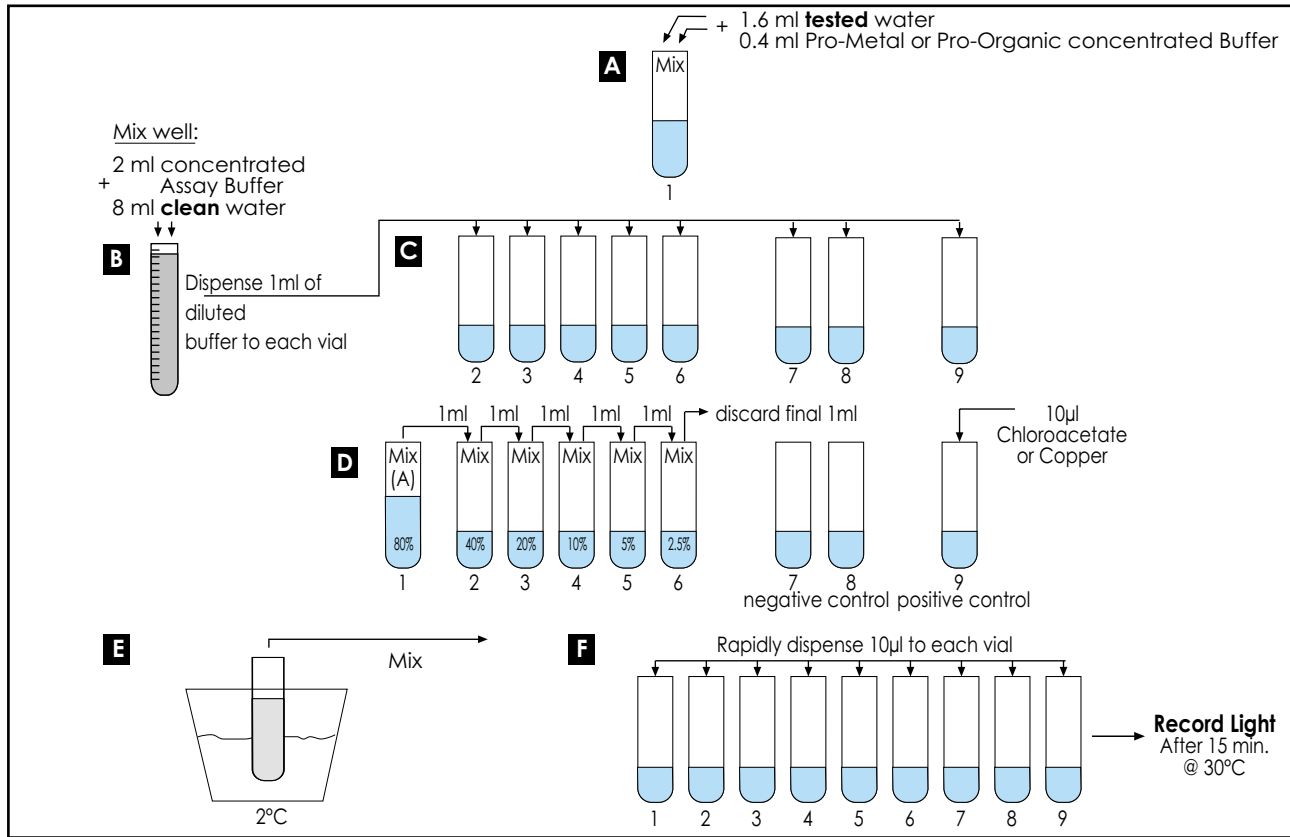
### Dilute the Water Sample

1. Mix tube #1 (containing the sample and buffer) well and add 1 ml to tube #2 (containing Assay Buffer). Mix well
2. Add 1 ml from tube #2 to #3 and mix well
3. Continue this dilution to tube #6
4. Discard 1 ml from tube #6. All tubes should now contain 1 ml
5. Place all tubes at 30°C for 5 minutes before dispensing the biosensor

### **Add Biosensor Incubate and Measure**

1. Remove the Biosensor solution from the refrigerator
2. Using the repeat dispenser add 10 $\mu$ l to each tube (including the controls). Mix well
3. Incubate the tubes in a water bath at 30°C for 15 minutes
4. Calculate the IC50 using the provided Excel module. For an example of an application of this procedure see - ***Using The Kit for Effluent or Highly Polluted River/Lake Toxicity Monitoring***, page 28.

# Protocol 1 – Determination of IC50



## Protocol 2- On-Site Testing

On site testing is usually performed in an emergency situation where contamination is suspected and rapid results are required.

### Equipment and Reagents Required

- A set of 6 tubes; 2 marked for tests, 2 for negative controls, 2 for positive controls
- 1.6 mL **local mineral** bottled water (standard clean water)
- 0.6 mL Pro-Metal and Pro-Organic concentrated Assay Buffers, each
- 0.8mL standard clean water + copper (dilute stock 1:100)
- 0.8 mL standard clean water + sodium chloroacetate (dilute stock 1:100)
- 60µl of hydrated bacteria maintained in Storage Buffer (from stock kept at 2°-4°C)
- Pipettors (repeat dispenser & regular) and tips
- Optional – portable incubator (catalog # SPOT-INC)
- Carrying case for outdoor testing
- Luminometer

### Solutions to Prepare Before Beginning the Assay

#### Negative Control

**Tube #1:** 0.8 mL of clean water and 0.2 mL concentrated Pro-Metal Buffer. Mix by pipetting up and down.

**Tube #2:** 0.8 mL of clean water and 0.2 mL concentrated Pro-Organic buffer. Mix by pipetting up and down.

### **Positive Controls**

**Tube #3:** 10 $\mu$ l of copper in 0.8mL clean water (control) and 0.2 mL concentrated Pro-Metal Buffer. Mix by pipetting up and down.

**Tube #4:** 10 $\mu$ l of sodium chloroacetate in 0.8mL clean water and 0.2 mL concentrated Pro-Organic buffer. Mix by pipetting up and down.

### **Test Samples**

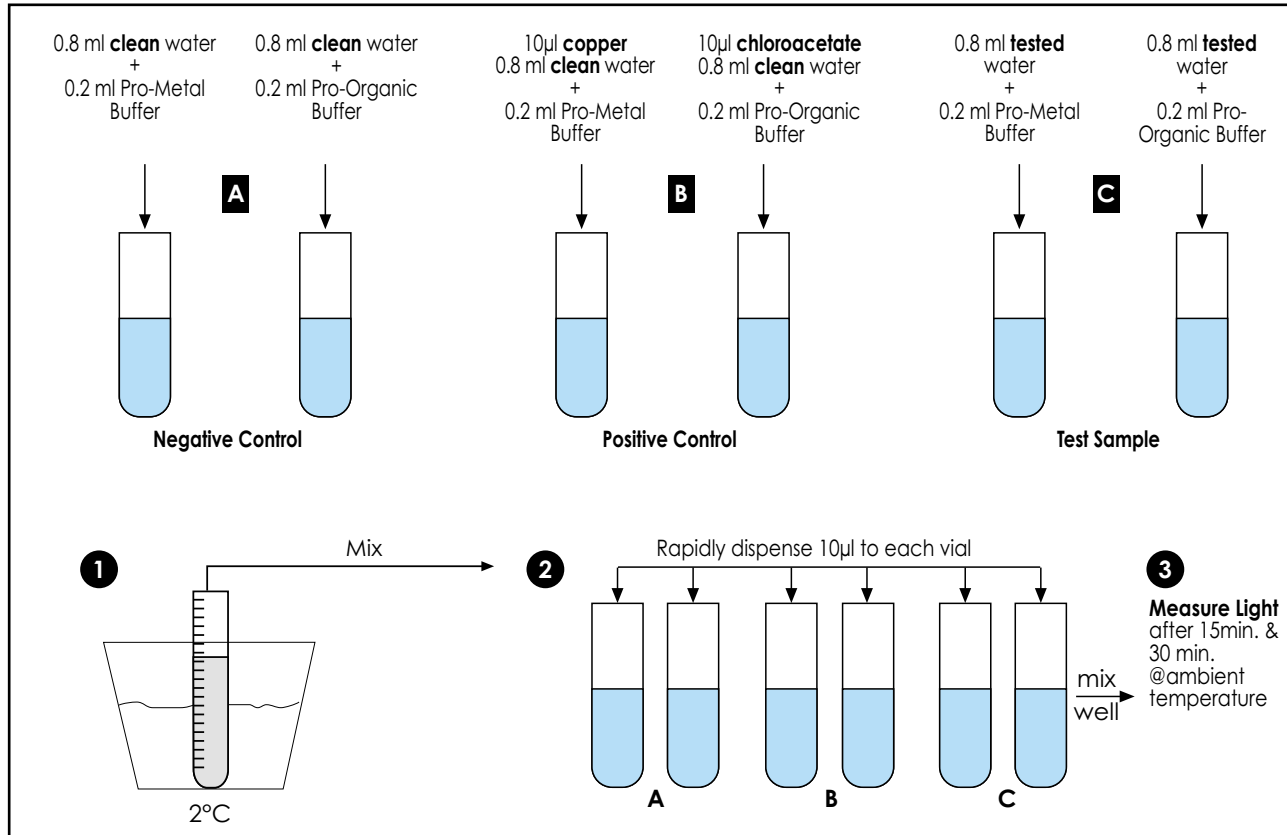
**Tube #5:** 0.8 mL of tested water and 0.2 mL concentrated Pro-Metal buffer. Mix.

**Tube #6:** 0.8 mL of tested water and 0.2 mL of concentrated Pro-Organic buffer. Mix.

### **Test Procedure**

1. Using the repeat dispenser rapidly dispense 10 $\mu$ L of the chilled suspended cells into each of the 6 test tubes. Mix well.
2. Incubate tubes at ambient temperature (preferably at 30°C).
3. Measure obtained luminescence in tubes after 15 min. & 30 min.
4. Compare to light level obtained in negative control. Use Excel module for calculations.

## Protocol 2 - On-Site Testing



## Protocol 3- Rapid Pre-Screening

Rapid pre-screening is used to test a number of different samples for a wide variety of organic compounds and metals. For an example of the use of this protocol see: Using The TOX-SCREEN<sup>3</sup> Test as Part of an **Early Warning System for Determining Dangerous Changes in Drinking Water Quality** page 29

### Equipment and Reagents Required

- A set of tubes marked O to test for organic compounds
- A set of tubes marked M to test for metals
- Pipettors (repeat dispenser & regular) and tips
- Heating plate & water bath (alternatively, temperature controlled air incubator)
- Optional – Vortex for mixing samples
- Luminometer
- Local mineral bottled water to serve as a negative control

### Solutions to Prepare Before Beginning the Assay

Prepare two sets (for organic [O] and metal [M] testing) of marked tubes. Designate 3 tubes from each set for controls. Put 0.8 mL of clean reference water into each of the 6 tubes.

#### Positive Control

Put 10µl of copper stock solution into one tube from the metal testing set.

Put 10µl of sodium chloroacetate stock solution into a tube from the organic testing set.

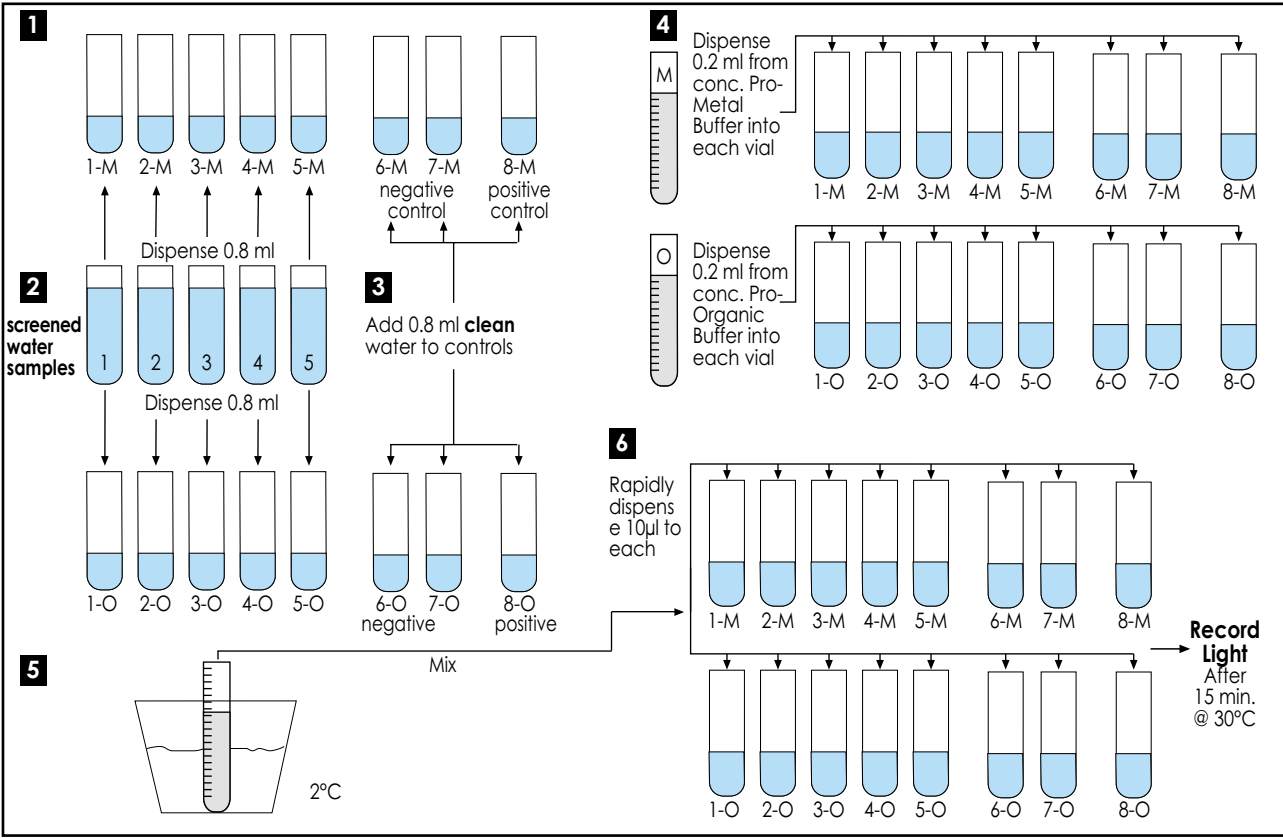
## Negative Control

Leave two tubes from each set as negative control samples.

## Test Procedure

1. Add water sample: dispense 0.8 mL of each tested water sample into one tube from each set.
2. Add Buffer: Add 0.2 mL of the concentrated Pro-Organic Buffer to all tubes (including controls) of the organic testing set, and 0.2 mL of the concentrated Pro-Metal Buffer to the second set of tubes (including controls).
3. Place all tubes at 30°C for 5 minutes before dispensing the biosensor
4. Add Biosensor: Remove the Biosensor solution from the refrigerator (maintained in water-filled tube). Rapidly dispense 10 $\mu$ L of the Biosensor into each tube. Mix well.
5. Incubate tubes in a water bath at 30°C.
6. Measure obtained luminescence after 15 min.
7. Determine the degree of relative toxicity for each water sample by calculating the extent of luminescence change as compared to the relevant negative control.

# Protocol 3 – Rapid Pre-Screening



## 4 Troubleshooting

<b>Problem</b>	<b>Possible Fault</b>	<b>Corrective action</b>
Inconsistent results	<ul style="list-style-type: none"><li>• Inaccurate pipetting of reagents</li><li>• Insufficiently mixed</li><li>• Inconsistent incubation time and temperature</li></ul>	<ul style="list-style-type: none"><li>• Make sure not to draw air bubbles</li><li>• Make sure to use a repeat dispenser to aliquot the bacteria</li><li>• Make sure to keep test conditions steady (e.g., incubation temperature, mixing, &amp; reading time)</li></ul>
Light decay of bacteria in storage buffer throughout the week is too steep	<ul style="list-style-type: none"><li>• Bacteria are not kept at 2-4°C</li><li>• Bacteria are left at room temperature for too long when preparing the test</li></ul>	<ul style="list-style-type: none"><li>• Make sure the refrigerator in which the bacteria solution is kept is at the right temperature</li><li>• Minimize the time the bacteria are exposed to room temperature</li></ul>
Positive controls are not working	<ul style="list-style-type: none"><li>• Improper dilution of the stock solution</li><li>• Bacteria have been exposed to sub-optimal conditions (e.g., high temperature, freezing)</li><li>• Inaccurate dispensing of bacteria</li></ul>	<ul style="list-style-type: none"><li>• Make sure the stock solution is diluted 1:100</li><li>• Replace bacteria stock with a new batch and make sure to maintain under proper conditions</li><li>• Use a repeat dispenser to aliquot the bacteria</li></ul>

# 5 Examples of the Use of the Kit

## Using The Kit as Part of an Early Warning System for Determining Dangerous Changes in Drinking Water Quality

### Introduction

This test is meant to act as a routine daily screen of a drinking water source. If the result exceeds the threshold the samples should be forwarded to chemical analysis to determine the exact nature of the contamination.

**Step 1** - collect water samples throughout the distribution network (for example, source, before treatment, after treatment, reservoirs, tanks, taps) and test them according to **Protocol 3**. It is recommended to test duplicates of each sample in order to reduce user errors. Since toxicity of a water source may vary with season, temperature, and time of day, take special care to carefully mark these variables in your log book.

For each water sample, determine:

**Question** - Is light inhibition greater than 50%?

**Answer 1** - YES – moderate to high level of toxicity - proceed to step 2.

**Answer 2** – NO – low to no detectable toxicity - proceed to step 3.

**Step 2** - Re-test the samples using **Protocol 1** to determine the degree of toxicity (i.e., how much can the sample be diluted and still exhibit 50% inhibition?)

**Step 3** – Keep using **Protocol 3** for routine measurements.

### **Day to day operation**

It is very important to establish a base line reading profile for each sampling point. This will enable the determination of a toxic event for each point once it occurs. Toward that end you will need to calculate the average reading of at least 15 data points from each sampling source. Next, determine the standard deviation (preferably  $3 \times \text{SD}$ ) to set the threshold level beyond which dangerous change in water quality is noteworthy.

In cases where toxicity threshold is exceeded, sample should be forwarded to chemical analysis in order to try and determine exact toxicity cause and nature.

See example below.

**Step 1** – determining the baseline of a given source water

Sample ID - 001 (post treatment plant) ; Assay Buffer – Pro-Organic

Date	Temp.	User ID	Tested vial	Luminescence (RLU)	Inhibitory Conc. (%)
12.1.07	25	John Doe	sample control	18562 22500	17.5
13.1.07	26	Jane Doe	sample control	16560 25400	34.8
14.1.07	25	Jane Doe	sample control	16681 21009	20.6
15.1.07	24	Jane Doe	sample control	21441 24845	13.7
17.1.07	24	John Doe	sample control	21745 27182	20
18.1.07	26	John Doe	sample control	20732 25439	18.5
19.1.07	23	John Doe	sample control	17399 21090	17.5
20.1.07	25	Jane Doe	sample control	18058 20998	14
23.1.07	24	Jane Doe	sample control	18514 20346	9
24.1.07	24	Jane Doe	sample control	18431 20897	11.8
25.1.07	23	John Doe	sample control	13168 19002	30.7
26.1.07	23	John Doe	sample control	12941 19879	34.9
27.1.07	24	Jane Doe	sample control	13629 18076	24.6
28.1.07	23	John Doe	sample control	16870 18934	10.9
2.2.07	21	John Doe	sample control	15717 18212	13.7
3.2.07	20	Jane Doe	sample control	11382 18010	36.8

**Average** 20.56  
**Standard Deviation** 9.14  
**Standard Deviation(3)** 27.42

Note: The shown values are for illustration only.

### Calculations:

The IC (Inhibitory Concentration) is calculated as:  $100 \times [1 - (RLU_{\text{sample}}/RLU_{\text{control}})]$ .

The threshold level is – 27.42.

### Step 2 – Routine Screening

Sample ID - 001 ; Assay Buffer – Pro-Organic

Testing Date	Temp. (°C)	Tester ID	Test	RLU	Relative Activity (%)	IC (%)
1.3.07	24	J.S	sample control	6500 8240	78.8 100	21.2 0
3.3.07	23	J.D	sample control	6100 8122	75.1 100	24.9 0
5.3.07	25	J.S	sample control	6813 8030	84.8 100	15.2 0
7.3.07	25	J.D	sample control	6771 8001	84.6 100	15.4 0
9.3.07	23	J.D	sample control	4930 7993	61.6 100	<b>38.3</b> 0

### Data Analysis

The sample tested on 9.3.07 exceeded the predetermined threshold level (IC=27.42%). It should be retested and if confirmed should be considered as suspected to contain a concentration of toxic chemical(s) above normal for that specific water source.

*Recommended reading: Water Security Initiative: Interim Guidance on Planning for Contamination Warning System Deployment (Office of Water EPA 817-R-07-002 May 2007).*

# Using the Kit for Effluent or Heavily Polluted River/Lake Toxicity Monitoring

## Introduction

Sewage treatment plants are based on the use of bacteria to breakdown the sewage. The bacteria may be harmed by toxic substances that enter the sewage plant. The kit is an ideal solution for monitoring the inflow of sewage treatment plants to avoid possible inhibition of microbial processes in the activated sludge due to toxic discharges.

Since each sewage plant has a different profile of inflow toxicity, the plant-specific threshold level must be determined (described below). Due to the high sensitivity of the test, and the concentrated nature of sewage it is usually necessary to dilute the tested water by up to a few hundred folds before determining luminescence inhibition profile. This dilution minimizes possible interference due to extreme pH, presence of suspended particles, colorants, etc.

The same logic applies to testing heavily polluted river/lake toxicity monitoring.

## Procedure:

1. Collect a few inflow samples and prepare a set of serial dilutions from each sample (e.g., from 10% down to 0.01% ; see scheme in test manual booklet).
2. Determine the dilution level that resulted in about 20% inhibition in luminescence (IC20).
3. For the next 2-3 weeks of normal plant operation, test the above-determined dilution and record degree of inhibition.
4. Determine the threshold level from the collected data according to the following equation:

Threshold level (in %) = Mean value + (Standard Deviation) x 3

Or,

Choose the highest inhibition level at which the plant functions properly.

5. Once determined, continue monitoring threshold level on a routine basis. Luminescence inhibition levels that exceed the threshold may mean that there is a dangerous toxic inflow that must be diluted before reaching the activation stage.

6. In addition, it is recommended to test the toxicity of the undiluted outflow for reliable monitoring of sewage treatment efficiency.

See example below.

Note: steps 1 and 2 need only be determined ONCE for each source.

## Step 1 – what dilution of sample exhibits 20% inhibition in luminescence?

Date - 12.12.00

Assay Buffer - Pro-Metal/Pro-Organic

Sample ID - 001

Time/Temp. - 10min/22°C

---

Vial no.	Sample conc. (%)	Luminescence (RLU)	Relative Activity (%)
1	10	350	4.1
2	5	1006	12.02
3	2.5	2450	29.2
4	1.25	5012	59.9
<b>5</b>	<b>0.62</b>	<b>6690</b>	<b>80</b>
6	0.31	6820	81.5
7	0.156	6905	82.5
8	0.078	7200	86
9	0	8750	100*
10	0	7980	

---

### Calculations:

Note: The shown values are for illustration only.

\* 100% activity is the reading when there are no toxic compounds present that may change light level. This figure is calculated by taking the average of the two negative controls (tubes 9 & 10)  $(8750 + 7980)/2 = 8365$ . Therefore, 8365 is defined as 100% activity.

**Relative Activity is calculated as:  $100 \times (\text{RLU}_{\text{sample}}/\text{RLU}_{\text{control}})$ .**

For example, in the tabel above Relative Activity for vial no.1 is calculated as :  $100 \times (350/8365) = 4.1\%$ . Etc.

In the example above - when the tested sample was diluted 161.3 folds (giving a final concentration of 0.62% = tube 5) it exhibited 80% Relative Activity, or, 20% inhibition.

This step only needs to be preformed once. For example, in this sewage plant all future assays will be performed with the water sample diluted to 0.62%

---

## Step 2 – what is the threshold level of the sample?

Sample ID - 001

Tested sample concentration – **0.62%**

Assay Buffer – Pro-Metal/Pro-Organic

Testing Date	Vial no.	RLU	Relative Activity (%)	IC(%)
1.1.01	1 - sample	6500	79.2	20.8
	2 - control	8206	100	0
3.1.01	3 - sample	6100	77.1	22.9
	4 - control	7910	100	0
5.1.01	5 - sample	6650	77.8	22.2
	6 - control	8540	100	0
7.1.01	7 - sample	6210	76.4	23.6
	8 - control	8120	100	0
9.1.01	9 - sample	6710	85	15
	10 - control	7890	100	0
<b>Mean</b>				<b>20.9</b>
<b>SD</b>				<b>3.39</b>
<b>Highest IC</b>				<b>23.6</b>
<b>Threshold (mean + 3SD)</b>				<b>31.08</b>

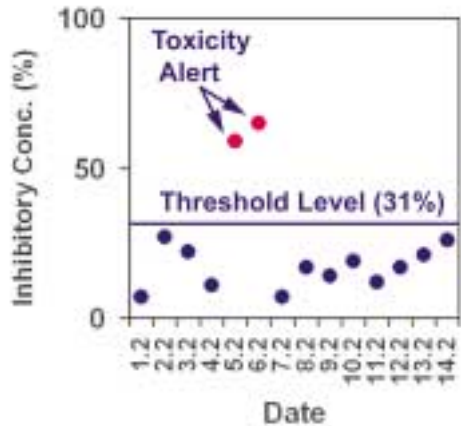
### Calculations:

The IC (Inhibitory Concentration) is calculated as:  $100 \times [1 - (RLU_{\text{sample}}/RLU_{\text{control}})]$ .  
Hence, the sample tested on 1.1.01 showed:  $100 \times [1 - 6500/8206] = 20.8\%$  inhibition. Etc.

### Step 3 – routine monitoring for changes in toxicity level

Date - 1-14.2.01  
Sample ID - 001

Tested concentration – 0.62%  
Assay Buffer – Pro-Metal/Pro-Organic



## 6 Frequently Asked Questions

### **Q: What is a toxicity test?**

**A:** A toxicity test can be considered a bioassay that allows measurement of damage. It is a measure of the degree to which a substance can elicit a deleterious effect (including death) in a given organism.

### **Q: How can luminous bacteria sense water toxicity?**

**A:** Luminous bacteria emit measurable light as a by-product of cell respiration. Chemophysical and biological factors that affect cell respiration, rapidly alter the level of luminescence. Similarly, factors that affect the cell's integrity, and especially membrane function, have a strong effect on in vivo luminescence. Hence, by simply comparing the luminescence level obtained in the suspected toxic sample with that obtained in the control (clean water sample), one may detect very low concentrations of a broad range of toxicants.

### **Q: What are the advantages of using a bioassay for environmental monitoring?**

**A:** Bioassays employ biological systems to detect toxicants in environmental samples (e.g., effluents, water, sediments, or soil) under investigation. The primary advantage of using bioassays is that toxicity can be evaluated. The use of bioassays provides a holistic approach that allows the toxicity evaluation of the total integrated effect of all constituent components, including toxicants and confounding variables, in a given complex sample matrix. The net assessment is the combined interactive evaluation of additive, antagonistic and synergistic effects of all sample components.

### **Q: Can the TOX-SCREEN<sup>3</sup> test replace chemical analysis?**

**A:** As a general rule, toxicity testing is never a substitute for chemical analysis. The test provides a rapid and sensitive tool for first response assessment of water contamination. An indication of a dangerous change in water quality should lead to a comprehensive analysis and/or emergency response.

**Q: How is CheckLight's toxicity test different from other bioluminescence-based tests?**

**A:** For most water toxicants tested, CheckLight's test was found to be many folds more sensitive than other bioluminescence-based tests. Unlike these tests, TOX-SCREEN<sup>3</sup> can be run at a wide temperature range (18°C-35°C). Moreover, a unique dual buffer set allows the discrimination between cationic heavy metals & metalloids and organic toxicants.

**Q: Are luminous bacteria dangerous? Do I need to be a trained microbiologist in order to be able to conduct the assays?**

**A:** Luminous bacteria are not pathogenic and are harmless. No special skill is required to carry out the different tests other than very basic laboratory techniques (pipetting, dilutions etc) and equipment (pipettor, tips, luminometer).

**Q: Why is there a control in each assay?**

**A:** Readings of the control are needed to calculate the relative luminescence inhibition by the sample toxicant. Fixing the reading from an unaffected control at 100% bioluminescence (0% toxicity) and reading the sample compared to it is the accepted method.

**Q: What should be the source of water used as reference?**

**A:** Use clean reference water that is the most similar to the tested water. For dilutions the use of double distilled water is recommended. DO NOT use de-ionized water as reference, as it occasionally contains traces of elements that can be inhibitory/toxic to bacteria.

**Q: Why is the light level in the Pro-Organic Buffer different than in the Pro-Metal Buffer?**

**A:** The composition of each buffer is unique. In addition, the Pro-Organic Buffer's pH is low (4.5) and that of the Pro-Metal is high (pH 7.5). Hence, the bacteria behave differently in each one of them.

**Q: Why is the light level in the negative control different on day1, day2, and day5?**

**A:** Once hydrated the freeze dried cells start emitting light. When stored at 2-4°C the decay in luminescence is maintained at a steady level, but still it is normal to observe about a 10 fold decrease in light with both assay buffers after 5-7 days. Their response to toxicants remains steady throughout the week.

**Q: How might chlorinated water affect luminescence?**

**A:** Chlorine is usually introduced into drinking water systems in order to avoid bacterial contamination. Since luminous bacteria used in the assay are also sensitive to this treatment, one should add sodium thiosulfate to the assay to dechlorinate the sample before adding the bacteria. When the bactericidal effect of chlorine is in question, samples with or without sodium thiosulfate may be used to evaluate the bactericidal activity of chlorine under the studied conditions.

**Q: What does the term EC50 mean and how do I calculate it?**

**A:** The degree of water toxicity is expressed in relative values, termed EC50 or IC50, that is defined as the minimal effective concentration of the tested water (in %) that results in 50% inhibition of the light level obtained in the clean water control sample under defined assay conditions. The provided Excel module assists you in automatically calculating this value from the generated data.

**Q: Can I “play around” with the volumes of bacteria, buffers and other assay conditions?**

**A:** No. It is extremely important to follow the test protocol instructions to the word. Since the test is very sensitive, any seemingly minor variations result in poor reliability.

**Q: Can I reuse the provided test tubes?**

**A:** Due to the high sensitivity of the assay, care should be taken to keep all tubes, plastic tips, and pipettes extremely clean. Do not reuse test tubes and do not wash glassware pipettors or pipette tips with detergent, acid, or solvents.

**Q: What is the shelf life of the reagents?**

**A:** The shelf life of the freeze dried bacteria is one year when stored in a deep-freezer (-10- -20°C). Reagent should not be stored in a self-defrosting freezer, which defrosts by warming up periodically. The assay buffers should be stored in a regular refrigerator (~4°C) and under no circumstances should they be frozen.

**Q: How do environmental conditions affect the response of the bacteria to toxic chemicals in water?**

**A:** While the optimal temperature for conducting the test is 30°C, the bacteria will respond well in a wide range of temperatures (18°-35°C). One should keep in mind that some chemicals effect bacteria faster than others, especially at sub-mg/L concentrations. As a rule of thumb, the lower the temperature the longer it takes for the assay to reach its maximal sensitivity (especially when testing organic toxicants). Under optimal conditions, an average time of 15 minutes is usually enough to detect most toxicants.





P.O.Box 72 Qiryat-Tivon 36000, Israel  
Tel: +972 4 9930530, Fax: +972 4 9533176  
[www.checklight.biz](http://www.checklight.biz)

---