CheckLight

Highlighting Water Safety

TOX SPOT 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.

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1-Introduction

About This Manual

This manual contains the instructions for how to use the TOX-SPOT 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 8, and find a list of problems that you may encounter in ***Troubleshooting*** page 17.

What Is the TOX-SPOT Assay?

The kit is for screening water from sources such as

- Groundwater
- Drinking water
- Surface 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- SPOT 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.

The common endpoint for toxicity tests is expressed in relative values, termed IC50 (Inhibition Concentration of 50%) that is defined as the minimal concentration of sampled water (in %) that results in 50% change in light output, as compared to light recorded in the clean reference water under defined assay conditions.

How Does the Kit Work?

The kit is based on using luminous bacteria as very sensitive and reliable biosensors. The operation principle is simple - changes in the level of luminescence indicate potential toxicity.

A starter kit which includes a carrying case holding all the required accessories is provided. Data is stored within the luminometer for later downloading and analysis. The testing procedure is simple as well: few simple steps that can be performed under varying field conditions. Typical applications include - mapping after pollution incidents/accidents; selection of samples for further/more expensive analysis; mapping to identify toxicity/concentration hotspots.

The tests can be used by emergency response teams, water companies, health and environmental supervising & monitoring authorities, municipal water system authorities, military units, etc.

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:

Reagents Provided

1. Biosensor - 25 stoppered vials holding freeze-dried luminous bacteria
2. Hydration Buffer
3. 75 Marked stoppered vials with pre-dispensed Pro-Metal concentrated Assay Buffer
   75 Marked stoppered vials with pre-dispensed Pro-Organic concentrated Assay Buffer
   25 Marked stoppered vials with pre-dispensed Pro-Metal concentrated Assay Buffer with positive control (copper).
   25 Marked stoppered vials with pre-dispensed Pro-Organic concentrated Assay Buffer
4. 200 Empty tubes + caps
5. Empty tube for storing clean water

Accessories (Supplied in starter kit only)

6. 0.1-1 mL pipettor and tips (SPOT-PIP; SPOT TIP)
7. Portable Luminometer (LUM-KK-01)
8. Temperature-controlled tube incubator (SPOT-INC)
9. Timer (SPOT-TIM)
10. Carrying case (SPOT-CAR)

Excel data analysis module

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

2 - Preparing For the Assay

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:

- Work in a clean manner to keep the reagents from getting contaminated

Do not:

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

Accuracy

Due to the high sensitivity of the kit, it is very important to add the reagents in exactly the right amounts and order. Therefore:

- 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 volume.
- 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 contains either no or sub-toxic amounts of the compounds that are being screened for. This negative control 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.

Positive Controls

To confirm proper performance of the test protocol, one should run a positive control test. Toward that end, two marked vials are provided -

One holding freeze-dried concentrated Pro-Organic Buffer and sodium chloroacetate, and the other holding freeze dried concentrated Pro-Metal Buffer and copper. When hydrated with clean water and spiked with the suspended bacteria, each should exhibit at least 50% reduction in light level, as compared to the negative control. If not, the test was not performed properly read protocol carefully, and repeat experiment.

Preparations

Before beginning an assay, prepare the following:

Biosensor

A freeze-dried preparation of the luminescent marine bacterium *Photobacterium leiognathi SB*.

Long-term storage - The shelf life of this reagent is one year when stored in a deep-freezer (-10°C – -20°C). Do not store the Biosensor in a self-defrosting freezer because it defrosts by warming up periodically. Do not store below -20°C.

Field storage - For optimal results, it is recommended to carry the freeze-dried biosensor in a chilled case (e.g., ice box) to the testing site. However, when that is not possible one could carry the vials inside the provided carrying case. Under such conditions, the freeze-dried bacteria will be very active for up for to 4 hours post-freezer removal, with diminishing activity in the following 4 hours. Un-used vials should be returned to the freezer and could be used again.

Preparing the Biosensor

Once reconstituted with Hydration Buffer, quickly and thoroughly mix the suspension by tapping on vial with finger. **The Biosensor suspension should be used after a 10 minute pre-incubation at 30°C.** Each individual vial contains enough material to test two samples (or a duplicate of one sample), together with one set of negative control and positive control with each Assay Buffer.

Buffers

Two concentrated (x5) Assay Buffers are provided - "Pro-Metal" to test for the presence of cationic heavy metals and metalloid compounds, and "Pro-Organic" to test for the presence of toxic organic compounds. The concentrated buffers are provided pre-dispended in freeze - dried form in individual marked vials.

Note that the texture of the freeze-dried material in the Pro-Metal vial looks like a white dense "cake", while in the Pro-Orgnic vial it looks more "Rubber-like" - that is normal. It is recommended to take two

aliquots of the sampled water and test with both Buffers in parallel. The Buffers are designed so that once diluted in water (pH range 6.0-8.5), the pH of the Pro-Organic Buffer is 4.5, and the pH of the Pro-Metal Buffer is 7.5. The shelf life of the Buffers is 12 months when stored in the freezer (-10°C to -20°C).

Also provided is Hydration Buffer for hydration of the suspended bacteria. It is stable for 12 months when stored at 4°C.

Dechlorination of Water Samples

The presence of chlorine and its by-products lead to rapid decay of bacterial bioluminescence. When required, up to 2 ppm chlorine could be chelated by adding sodium thiosulfate (2ppm). Same concentration to the controls.

Note that one could order a set of Assay Buffers (Pro-Metal & Pro-Organic) that already contain thiosulfate. Please inquire for details.

Higher concentrations of chlorine should be properly diluted in clean water prlor to addition of thiosulfate, or removed by other means. Keep in mind, though, that thiosulfate interacts with some toxicants, such as cyanide, mercury and lead, at certain concentrations, and thus reduces the overall observed toxicity of the sample. The degree of effect will depend on the residual chlorine level.

3- Assay Procedure

Outline of the Assay Procedure

The assay is performed in the following steps:

- Turn on the incubator
- Prepare all reagents and equipment
- Collect the sample
- Prepare and incubate the Biosensor Bacteria
- Add water sample & negative control water to the freeze-dried buffers vials
- Add Biosensor suspension
- Incubate
- Read results
- Interpret results

Equipment and Reagents Required

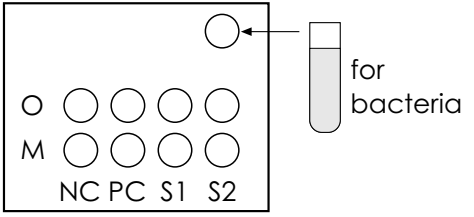
For TWO water samples (or a duplicate of one sample) you will need:

- 8 empty tubes with caps
- 3 vials with Pro-Metal concentrated Assay Buffer (freeze-dried)
- 3 vials with Pro-Organic concentrated Assay Buffer (freeze-dried)
- 1 vial with copper in Pro-Metal concentrated Assay Buffer (freeze-dried)
- 1 vial with sodium chloroacetate in Pro-Organic concentrated Assay Buffer (freeze dried)
- 0.9 ml Hydration Buffer
- One vial of freeze-dried bacteria
- Pipettor and tips
- Incubator
- Luminometer
- 3.6 ml local mineral bottled water to serve as a negative control

Turn on the Incubator

- Connect incubator plug to the car's cigarette lighter while the engine is running. Turn on the incubator (it is pre-set to 30°C. warming up could take up to 30 minutes. It is recommended to perform this step while driving to the testing site).

Position 8 plastic tubes with caps in the incubator as shown in the following diagram:



N = Negative control
P = Positive control
S = Sample
M = Metal
O = Organic

Add Hydration Buffer to the Biosensor:

- After the incubator has reached 30°C, add 0.9 ml Hydration Buffer to a vial of freeze-dried bacteria.
- Mix gently by tapping vial with finger.
- Place the vial in the incubator for 10 minutes.
- Set the timer to 10 minutes.
- Place 8 freeze-dried vials in the designated slots in the gray foam. Make sure you have a (2x4) set composed of: (NC, PC, S1, S2) M & (NC, PC, S1, S2) O.

In the first runs, if you are not sure that you will conclude all the preparations (dispensing buffers, controls, and samples) within 10 minutes, delay the incubation step until all is ready.

Note - It is very important to dispense the hydrated bacteria immediately following 10 minutes incubation at 30°C.

Hydration of freeze-dried Buffers

- Remove and discard cap and add 0.9 ml clean water to each of the following vials -
  M (NC, PC)
  O (NC, PC)

Mix contents well by flicking with finger.

Dispense Test Samples

- Add 0.9 ml from the 1st sample to vials S1-M and S1-O. Mix well by flicking with finger.
- Add 0.9 ml from the 2nd sample to vials S2-M and S2-O. Mix well by flicking with finger.

Dispense Biosensor Solution

- Remove the biosensor from the incubator and mix well by flicking with finger.
- Quickly add 0.1 ml to each of the eight vials.
- Carefully transfer the content of each vial to its plastic tube counterpart.
- Place black cap on tube. Mix contents rapidly by shaking up and down.
- Set the timer to 15 minutes.
- Incubate all 8 plastic tubes at 30°C for 15 minutes.

Read the Results

- With the Luminometer in an upright position, starting with the Metal set, insert each tube and record the luminescence level. This step should be carried out as **quickly as possible**, as light emission keeps changing with time.

Note - The light level in the metal control is higher than that obtained in the organic control - that is normal.

Analyze the Data

Record each luminescence level reading into the provided Excel spreadsheet to automatically calculate the toxicity levels.

Negative control (NC) is defined as 100% and the Positive control (PC) should not be less than 50% of the NC. For instance, if the NC reading is 400 and the PC reading is 350 the assay results are invalid.

An Inhibitory concentration of more than 50% in a tested sample indicates toxicity.

Note that although most toxic chemicals exert their effect by reducing luminescence, there are some exceptions that result in luminescence increase. Hence, a 50% increase should also be regarded as a Toxicity Alert.

4-Troubleshooting

Problem	Possible Fault	Corrective action
Negative control exhibits zero light	• Bacteria were hydrated with the wrong buffer • Bacteria were exposed to extreme temperatures (e.g., freezing or >40°C)	• Read the instructions carefully and repeat the test with a new vial
Light level in tested sample in Pro-Organic Buffer is 2 times that of the negative control	• The pH of the tested sample is higher than 8.5	• The buffer capacity of the concentrated Pro Organic Buffer is designed to maintain final pH of 4.5 of samples with pH range of 6.0-8.5. Adjust the pH of the sample accordingly before testing.
Inconsistent results	• Inaccurate pipetting of reagents • Insufficiently mixed • Inconsistent incubation time and temperature • Long exposure of freeze-dried biosensor to changing environmental conditions.	• Make sure to keep test conditions steady (e.g., incubation temperature, mixing, & reading time). • Minimize the exposure of the biosensor to ambient temperature. • Make sure to use Biosensor exactly 10 minutes after hydration and incubation at 30°C.
Positive controls are not working	• Inaccurate dispensing of bacteria • Inaccurate dispensing of water	• Make sure not to draw air bubbles with the tip. • Make sure you follow the exact order of adding the reagents • Make sure to keep test conditions steady (e.g., incubation temperature, mixing, & reading time). • Minimize the exposure of the biosensor to ambient temperature.

5 - Frequently Asked Questions

Q: What is a toxicity test?

A: A toxicity test can be considered a bioassay that allows measurement of damage to living cells. 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-SPOT 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 the TOX-SPOT test different from other bioluminescence-based tests?

A: For most water toxicants tested, CheckLight's test was found to be many times more sensitive than other bioluminescence-based tests. In addition, a unique dual buffer set allows for the discrimination between cationic heavy metals and organic toxicants.

Q: Are luminous bacteria dangerous? Do I need to be a trained microbiologist in order to be able to conduct CheckLight's 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.

DO NOT use de-ionized water as negative control, 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: 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°C to -20°C). Freeze-dried 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.

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