

## Frequently Asked Questions – TOX-SPOT

### **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, promptly 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 ToxScreen 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. □ Moreover, a unique dual buffer set allows the discrimination between cationic heavy metals and organic toxicants.

TOXSCREEN CAL VER 1.0

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□□

**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? what water source should I use as reference?**

**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.

The water used as clean water control should be as similar as possible to the tested water. Local mineral water could be used, but not double distilled or deionized water.

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**Q: How might chlorinated water effect 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.

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**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 software 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.

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

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**A:** Due to the high sensitivity of the assay, care should be taken to keep all vials, plastic tips, and pipettes extremely clean. Do not reuse test vials 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 (-14°C +/-5°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 effect 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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