Rapid Screening Toxicity Test with LumoStix™ Environmental Assessment Kit

Introduction

This method employs a novel, patent pending sampling and test device called the LumoStix™ to rapidly screen samples for toxicity in the field. Swabs at each end of the device provide both a sampling and test location and the prepared BioTox™ reagent is employed to assess toxicity using a portable luminometer. The LumoStix™ device is easy to use, and can be adapted for a variety of sample applications. This protocol can be employed for pond, lake, river or sea waters.

Principle

The inhibition of light emission of photobacterium *Aliivibrio fischeri (*formerly *Vibrio fischeri)* from direct contact to sample toxicants is determined by measuring light emission from bacteria on the surface of the swab at one end of the device after sample exposure and comparing it to the light emission of an analogous amount of bacteria on the swab at the other end of the device representing background levels of bioluminescence. Inhibition of light emission after an incubation period compared to the background indicates sample toxicity. The assay is therefore entirely portable and uses minimal equipment. Inhibition of light output can be calculated manually or with appropriate software.

Reagents and Equipment

Reagents

- 1243-300 BioTox™ LumoStix™ Instant Kit

1243-300 contains

- 5 vials of BioTox™ bacteria
- 1 Bottle of Reagent Diluent
- 20 LumoStix™ devices
- 5 Pasteur pipettes

Instrumentation

- Kikkoman PD30 Luminometer

Procedure

Reconstitution of the Reagent

Reconstitute the BioTox™ reagent by adding approximately 1.0 mL of Reagent Diluent cooled to 4 °C to one vial of lyophilized reagent. The reconstituted reagent is immediately ready for use and should be used within 2 hours of rehydration.

Sample Preparation

1. Open the end of the LumoStix™ device marked **Reagent**. Dip the swab in the rehydrated reagent.
2. Close the device
3. Open the end of the device marked **Sample**. Dip the swab to the rehydrated reagent.
4. Pipette approximately 400 µL (8 drops) of unknown sample into the sample vial.
5. Add 50 µL (one drop) of 20 % NaCl solution to the sample vial to adjust the salinity. Mix liquid with the pipette.
6. Reattach the vial tightly to the end of the LumoStix™ device.
7. Incubate for 30 min in an upright position with the **Sample** end down.
8. Measure sample light emission by inserting the **Sample** end of the LumoStix™ device into the PD30 luminometer.
9. Once the light reading has been acquired remove the device from the luminometer.
10. Turn the LumoStix™ device upside down very carefully.
11. Insert the **Reagent** end of the device into the luminometer and measure background (reagent only) light emission.
12. Once the light reading has been acquired remove the device from the luminometer.
13. Calculate results manually or with a software program. If sample luminescence is less than 50% of the reagent luminescence, the sample contains compounds that are toxic to the test bacteria.
14. Discard the device.

Procedural Notes

The *Aliivibrio fischeri* reagent volume in both Reagent and Sample end of the device is equal. Due to the specially designed light collection optics of the PD30, the small sample liquid volume has no effect on the total collected light if the sample has no colour or turbidity.

Ensure that seals are tight when reconnecting the device. Carefully turn the device upside down (Do not shake or drop) after measuring the Sample

luminescence before measuring the Reagent luminescence.

Plan experiments to use all reconstituted reagent. Each vial of reagent can accommodate at least 4 sample measurements. Once reconstituted, the reagent must be used and **cannot** be stored.

It is recommended to carry out all experiments between 15 °C and 25 °C if possible.

If you have any questions regarding the procedure or product quality, contact a representative at www.biotoxicity.com

References

1. Lappalainen, J., Loikkanen, S., Havana, M., Karp, M., Sjöberg, A.-M. and Wirtanen, G. (2000). Microbial Testing Methods for Detection of Residual Cleaning Agents and Disinfectants-Prevention of ATP Bioluminescence Measurement Errors in the Food Industry. J. Food Prot., Vol. 63, No. 2, pp. 210-215.pp. 210-215.

Calculations

Tox% = 100% x (RLU_{sample} / $RLU_{reagent}$)

Where:

RLU_{sample} = Luminescence intensity of **Sample** after contact time of 30 min in RLU.

$RLU_{reagent}$ = Luminescence intensity of **Reagent** in RLU.