TOXI - SCREENING KIT

BENCH PROTOCOL

PRINCIPLE AND FEATURES

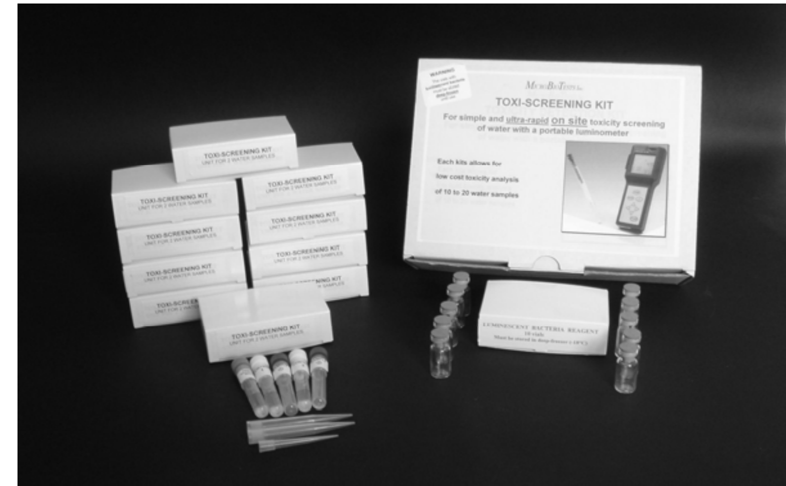
The Toxi-Screening Kit is a simplified “field modification” of bioassays which measure the decrease in luminescence in specific (mostly marine) bacteria producing bioluminescence as a by-product of their cellular respiration.
The amount of light produced by the bacteria is proportional to the intensity of cellular respiration and is measured in a portable luminometer in Relative Light Units (RLU).
Inhibition of the bacterial respiration under toxic stress automatically leads to decrease of the bioluminescence and this decrease of luminescence in the water sample is measured after a short exposure time (30 minutes) and compared to the decrease in luminescence in a (non-toxic) control water.
The magnitude of the decrease in luminescence in the analysed water versus that in the control water is an indication for the degree of toxicity of the water sample.

This very simple and practical assay is suited for a variety of analyses such as toxicity screening of surface waters, ground waters and leachates of solid wastes, routine toxicity screening of drinking waters, water contamination emergencies, and monitoring of the efficiency of various kinds of detoxification treatments of aquatic and terrestrial environments.

The Toxi-Screening test method is a “single shot” bioassay on non-diluted waters, without preparation of dilution series.
The assay can be performed anytime, anywhere in the field, in a temperature range of 15°C to 25°C.

The measurements are performed in a handy portable luminometer, included in a “Luminescence Measurement Case”, which also contains various other items for easy performance of the assays “in field conditions”.
N.B. : The Luminescence Measurement Case also contains specific items for ultra-rapid evaluation of the degree of microbial contamination of waters with the aid of the “Bacterial Contamination Screening Kit”.

The Toxi-Screening Kit (see Figure) is composed of one box containing 10 vials of freeze-dried *Vibrio fischeri* luminescent bacteria (which must be stored at -18°C prior to use), and of 10 boxes containing tubes and reagents, each allowing 2 assays on a suspect water. The freeze-dried bacteria and the reagents have a shelf life of 6 months to 1 year.
Performance of the assays is very simple and only takes 1 hour of time, i.e. 30 minutes for rehydration of the freeze-dried bacteria, followed by 30 minutes exposure to the suspected water sample in parallel to a control water, prior to measurement of the decrease of luminescence.



TEST PROCEDURE

Transfer of the materials to the Luminescent Measurement Case
On arrival in the field, open one box “Unit for 2 water samples” and place all the items in the holes located in the upper left corner of the Luminescence Measurement Case.
Take one vial with freeze-dried bacteria and place it in the (largest) of the 6 holes.

Rehydration of the freeze-dried bacteria

1. Put one 1 ml finntip on the fitting of the 1 ml Finnpipette and transfer the total contents of the Osmotic Adjument Medium tube into the vial with bacteria.
2. Close the vial with bacteria, shake the contents thoroughly and put the vial back into its place in the Luminescence Measurement Case.
3. Set the timer 30 minutes.

Sampling
*N.B.: An analysis can be made either on two separate water samples, or alternatively the assay can be performed “in duplicate” on only one water sample to check the precision of the first measurement.
After rehydration of the bacteria, the two analyses must, however, be performed within a few hours.*

1. Put the second 1 ml finntip on the fitting of the 1 ml Finnpipette and take a 1 ml water sample for transfer into the Sample 1 tube.
2. Take again a 1 ml water sample (either from the same water, or from a different water – in the latter case after rinsing the finntip) and transfer it into the Sample 2 tube.

N.B. : The Sample tubes already contain a (very small) volume of liquid to adjust the osmotic pressure of the water sample to the appropriate value for the (marine) Vibrio fischeri bacteria.

Transfer of the luminescent bacteria in the Sample tubes and the Control tubes

1. Put the 200 µl finntip on the fitting of the 200 µl Finnpipette.
2. After 30 min rehydration, shake the vial with the bacteria and transfer 200 µl of the bacterial suspension in the two Sample tubes and the two Control tubes.

N.B. : The Control tubes already contain a specific volume of non-toxic water, already adjusted to the right osmotic pressure for the bacteria.

3. Close the tubes, shake the contents thoroughly and put the tubes back into their respective holes in the Luminescence Measurement Case.
4. Set the timer again to 30 minutes.

Measurement at time t0

1. Take the luminometer and switch it on by pushing the “Power” button. Wait till the instrument has calibrated itself by a 10 seconds countdown, visible on the display.
2. Remove the caps from Sample 1 and Control 1 tubes and adjust the tubes tightly to the 2 holders present in the Luminescence Measurement Case.
3. Open the lid of the luminometer, insert Control tube 1 with its holder and close the luminometer.

N.B. : The luminometer must always be kept vertically when containing a tube in its holder !

4. Push the “Enter” button and wait for the 10 seconds countdown to see the luminescence score on the display.
5. Score the luminescence figure (in RLU) on the Results Sheet.
6. Take the tube and the holder out of the luminometer, separate the tube from the holder, close the tube with its cap and put it back into its place in the Luminescence Measurement Case.

N.B. When a tube is kept for more than 30 seconds in the luminometer, the instrument will give beep signals indicating that the tube must be removed !

7. Repeat the former operation with Sample tube 1 and subsequently with Control tube 2 and Sample tube 2, and score each time the respective luminescence figures.

Measurement at time t30

After 30 minutes exposure of the luminescent bacteria to the 2 control waters and the 2 sample waters, repeat all the operations indicated above for the measurements at t0, for a second series of measurements at t30 minutes.
All the tubes must be shaken again prior to the measurements.

Data evaluation

During the 30 minutes exposure time, the intensity of the bacterial luminescence will normally decrease, so the t30 RLU value will be lower than the t0 figure for both the water sample and the control water.
In case of toxic stress, the decrease in luminescence in the tube with the water sample will be more pronounced than that in the control water.

The degree of toxic stress can be calculated as the ratio of the magnitude of the luminescence decrease in the water sample, versus that in the control water.

The percentage toxicity of the water sample can be calculated with the formula :

$$\% \text{ toxicity} = \frac{(\text{RLU at t0} - \text{RLU at t30})\text{ sample}}{(\text{RLU at t0} - \text{RLU at t30})\text{ control}} \times 100$$

Important remarks

The toxic responses of any kind of bacterial luminescence inhibition assay are dependent not only of the concentration of the toxicants present, but also of the “chemical” composition of the water, i.e. the amount of “salts and ions” present in the water.
As a general rule it appears that the higher the conductivity and the hardness of the water, the lower the sensitivity for toxic chemicals and it is therefore strongly advised to make in parallel with the Toxi-Screening Test, a measurement of the conductivity and/or the hardness of the analysed water samples.

Like for any bioassay with other test species, the toxic response given by the Toxi-Screening test with the luminescent bacteria is also “chemical dependent” and will range from a very high sensitivity for particular compounds (a few ppb) to a low sensitivity (ppm) for others.

The former facts must therefore always be taken into consideration for a correct evaluation of the findings !

As a general rule of thumb, samples with a toxicity percentage of 20-25% clearly signal the presence of toxic compound(s) which should trigger further attention.