

DECHLORINATION OF WATER SAMPLES

The presence of chlorine and its byproducts leads to decay of bacterial luminescence. When water sample had been chlorinated, up to 1 ppm chlorine interference can be removed by the addition of sodium thiosulfate (10 ppm). Higher concentrations of chlorine should be properly diluted in pure water prior to the addition of thiosulfate.

ASSAY PROCEDURE

A. Determination of IC₅₀

1. Prepare the original sample for the toxicity assay by preparing the following dilutions:
 - a) Prepare the sample dilution series in a test tube according to the scheme below, by using **Distilled water** as a diluent.

NOTE: Procedure listed below is done with freshwater or low salinity samples. For samples with high salinity, reduce the NaCl amount and omit step 4 or contact Abraxis technical support.

NOTE:

- Dilution 0, no dilution required (dilution factor at final dilution will be 1:1.25).
 - Dilution 1, 2 mL sample and 2 mL of **Distilled water** (dilution factor at final assay will be 1:2.5).
 - Dilution 2, for example 2 mL of sample (dilution 1) + 2 ml of **Distilled water** (dilution factor at final assay will be 1:5), mix.
 - Dilution 3, for example 2 mL of sample (dilution 2) + 2 mL of **Distilled water** (dilution factor at final dilution will be 1:10), mix.
 - Dilution 4, for example 2 mL of sample (dilution 3) + 2 mL of **Distilled water** (dilution factor at final dilution will be 1:20), mix.
 - Dilution 5, for example 2 mL of sample (dilution 4) + 2 mL of **Distilled water** (dilution factor at final dilution will be 1:40), mix.
 - Dilution 6, for example 2 mL of sample (dilution 5) + 2 mL of **Distilled water** (dilution factor at final dilution will be 1:80), mix.
2. Reconstitute the **Vibrio fischeri Reagent** by adding 2.5 mL of +4°C **Reconstitution Solution**. The reconstituted reagent should be equilibrated at +4°C for at least 30 minutes. **The reconstituted reagent must be used within the same day and it can not be frozen.**
 3. Into duplicate test cuvettes, Pipette 800 uL of: a) distilled water (blank), b) distilled water (negative control), c) 800 uL of positive control, and d) each sample dilution. Temperate all controls, samples and dilutions to +15 °C for at least 15 min. Keep all controls, samples and dilutions at +15 °C during the whole measurement. Suitable dilutions are chosen depending on the expected toxicity of the sample. The D = 0 dilution level is used for samples with very low toxicity.
 4. Pipette 100 ul of **Osmotic Adjusting Buffer** into all cuvettes, mix well.
 5. Pipette 100 ul of the final bacterial suspension into the cuvettes set up on step 3 b to d, **do not add bacteria** to cuvette used for reagent blank. It is recommended to perform all dilutions and controls as duplicate samples.
 6. Incubate at +15 °C for at least 15 minutes (suggested incubation and reading times are 15, 30, 60 minutes)
 7. After proper incubation time, measure the average luminescence intensity (I₀) from the cuvettes containing bacterial suspension and labelled negative control (cuvettes 1, 2). Determine the average luminescence intensity (I_t) from the negative control and each sample. Repeat readings for all samples at a later time interval i.e. 30, 60 minutes using exactly the same time interval as during the first measurement.

NOTES:

- Temperature changes during the measurement may affect the results. Ensure that all reagents have reached the same temperature (+15 °C).
- The contact time should be exactly the same for all the samples and the controls. The most commonly used contact times are 15, 30 and 60 minutes.

B. Rapid Toxicity Screening

1. Reconstitute the **Vibrio fischeri Reagent** by adding 2.5 mL of +4 °C **Reconstitution Solution**. The reconstituted reagent should be equilibrated at +4°C for at least 30 minutes by placing in incubation chamber. **The reconstituted reagent must be used within the same day and it can not be frozen.**
2. Into test cuvettes, Pipette 1000 uL of: a) distilled water (blank), b) distilled water (negative control), c) 1000 uL of positive control, and d) each sample. Temperate all controls, samples and dilutions to +15 °C for at least 15 min by placing in incubation chamber. Keep all controls, samples and dilutions at +15 °C during the whole measurement.
3. Pipette 100 ul of **Osmotic Adjusting Buffer** into all cuvettes, mix well.
4. Pipette 100 ul of the final bacterial suspension into the cuvettes set up on step B.2 b to d, **do not add bacteria** to cuvette used for reagent blank. It is recommended to do all dilutions and controls as duplicate samples.
5. Incubate sample dilutions at approximate +15 °C in incubation chamber for 10 to 20 minutes. Determine the luminescence intensity and the degree of relative toxicity for each tested sample by calculating the extent of luminescence inhibition as compared to the negative control. Repeat for all samples using exactly the same time interval as during the first measurement.

CALCULATION OF RESULTS: IC₅₀ Determination

Calculate the inhibition percentage (INH%) for each sample and dilutions as follows (in this example the contact time is 15 min.):

$$\text{INH\%} = \frac{I_{15}}{I_0} \times 100$$

Where:

$$\begin{aligned} I_{15} &= \text{Luminescence intensity of test sample after contact time (15 min) in RLU.} \\ I_{0-15} &= \text{Luminescence intensity of the negative control (15 min) in RLU.} \end{aligned}$$

The EC₅₀-value is determined by using standard linear regression analysis. If the range of value pairs can not be linearized, the EC₅₀-value can be determined graphically using a double logarithmic co-ordinate system. The INH% is plotted on the y-axis and the concentration (in mg/l, mol/l or % of original sample) on the x-axis.

QUALITY CONTROL

Replicate readings between cuvettes for each control and samples should be within 20% of each other.

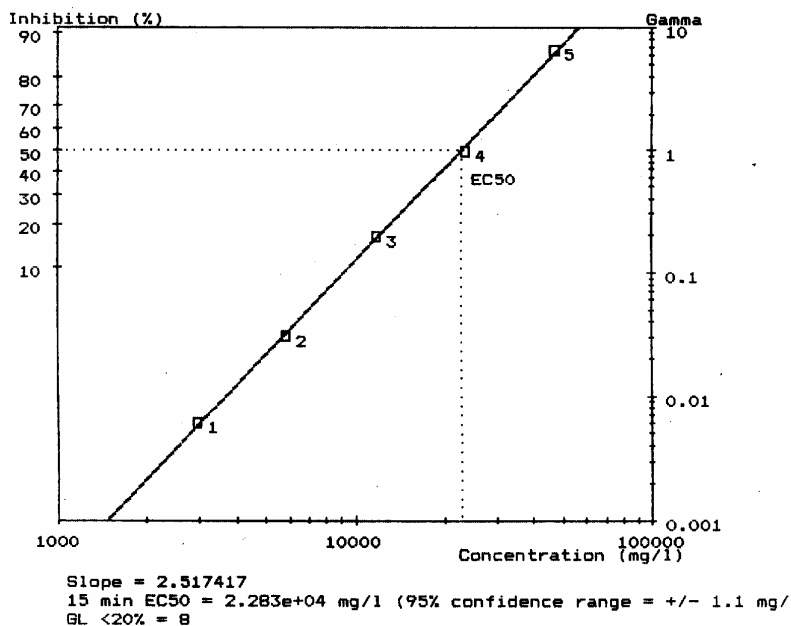


Figure 1: Toxicity of ethanol



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AbraTox Kit Instructions for use

INTENDED USE

The **AbraTox Kit** is used for the determination of toxicity of water soluble samples. The inhibitory effect of the sample on the light emission of luminescent bacteria, *Vibrio fischeri*, is measured with a luminometer or with our cost-effective **AbraTox-Cam** detection system. The **AbraTox Kit** provides a rapid, easy to use method for measuring toxicity of aqueous samples such as industrial effluents, septic discharge; extracts from sediments and soil; surface, ground and drinking water, etc.

ASSAY PRINCIPLE

The inhibition of the luminescence is determined by combining different dilutions of the test sample with luminescent bacteria. The decrease of light intensity is measured after a contact time of 15 - 60 minutes. The inhibitory effect of dilutions is compared to a toxin free control to give the percentage inhibition (INH%). The value is plotted against the dilution factor and the resultant curve is used to calculate the EC₅₀ (Effective Concentration giving 50% inhibition of light output) of the sample or the extent of luminescence inhibition as compared to the negative control when using the rapid toxicity screen procedure.

KIT CONTENTS AND STORAGE

1. *Vibrio fischeri* Reagent (PN300107), 4 vials. Lyophilized *Vibrio fischeri* together with stabilisers.
2. Reconstitution Solution (PN300108), 2 vials, 12.5 ml.
3. Osmotic Adjusting Buffer (AOB) (PN300109), 1 bottle, 50 ml.
4. Positive Control (PN300110), optional
5. Negative Control (PN300111)

Reagent storage

The stability of the reagents is guaranteed until the expiry date if stored at -18 °C. The Kit should be stored at <= -18 °C in a frost free freezer.

For laboratory use only. Not for drug, household or other use.

General Limited Warranty: Abraxis LLC 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. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

REAGENTS AND INSTRUMENTS NEEDED

AbraTox Kit (PN500091)

- Luminometer (required sensitivity of at least ~ 1 fmoles ATP equivalent/mL) or
- **AbraTox-Cam** (special designed camera for the detection of luminescence) PN 475041
- +15 °C incubator (PN 475051 or equivalent)
- Luminometer cuvettes (PN003056) or equivalent
- Pipettes capable of delivering 100 µl, 0.8 mL (IC₅₀ procedure) 1 ml (rapid screening procedure) and 2.5 ml pipettes and pipette tips
- Empty test tubes or vials for dilutions
- Distilled Water (PN 500092) (for sample dilutions optional)
- Sodium Thiosulfate solution (PN500093) (optional for chlorinated samples)