# BioTox™ LumoPlate™ Ultimate Matrix Kit Procedure

## Introduction

The BioTox™ Ultimate Matrix Kit is a cost-effective new assay format to determine toxicity in water soluble samples based on ISO Standard 21338. Bioluminescence inhibition from sample constituents on a strain of *Aliivibrio fischeri* bacteria (formerly *Vibrio fischeri*) is measured by a microplate luminometer. The kit has been designed to provide rapid, reliable results for measuring acute toxicity directly in waste water samples, sediments, dissolved pure substances and samples in coloured or turbid matrices. The microplate format and kinetic measurement method permits high throughput analysis of several sample simultaneously while correcting for matrix interferences automatically.

## Principle

Sample toxicity to photobacterium *Aliivibrio fischeri* is measured by comparing initial and final light emission after a predetermined contact time (5-30 min). Bacterial bioluminescence is enzymatically controlled and sample components that alter these processes cause decreases in light intensity. Emission maximum intensity (peak value) occurs within 5 s of mixing and is measured and recorded immediately after the bacterial suspension is added to the sample. Emission is again measured after the incubation period to determine signal reduction. A series of sample dilutions provide concentration-dependant differences in inhibitory effects used to calculate $EC_{50}$ values ($EC_{50}$ = effective concentration causing 50% inhibition of light output).

## Kit Contents
**1243-700 contains:**

- 1243-157 - 10 vials *Aliivibrio fischeri* bacterial reagent
- 1243-110 - 10 bottles x reagent diluent (12.5 mL)
- 1243-115 – 1 x 50 mL bottle of OAS solution
- 1243-125 – 2 x 250 mL bottles of Sample Diluent
- 1243-PLT – 10 x sterile white 96-well microplates

## Other Required Materials

- Microplate luminometer (contact EBPI for pricing and available model)
- cooling block (15 °C) may be included with luminometer
- pipette 10 – 5000 L and disposable pipette tips (20 – 5000 µL)
- pH meter (accuracy 0.1 pH units)
- $O_2$ probe (if available)

## Storage

Bacterial reagents are shipped lyophilized with stabilizers and are guaranteed until the best before date if stored at -18 °C. Reagent Diluent, Sample Diluent and OAS solution should be stored at 4 °C upon receipt.

## Warranty

The BioTox™ LumoPlate™ Ultimate matrix kit or any of its components will be replaced if defective in manufacturing or packaging. Liability is restricted to replacement of kit materials. Complete results of tests should accompany all replacement claims. Visit www.biotoxicity.com for more information.

## Assay Procedure (Refer to ISO 21338:2010 for extra instructions)

## Reagent Preparation

1. Reconstitute the *Aliivibrio fischeri* reagent (1243-157) by pouring one vial of cooled (4 °C) reagent diluent (1243-110) into the lyophilized bacteria. Equilibrate the reconstituted reagent to 4 - 6 °C in a refrigerator for at least 30 minutes. The reagent is now ready for testing and should be used within one day. Before the assay, stabilize the reagent at 15 °C for another 30 minutes.

2. Ensure that Aliivibrio fischeri reagent is reconstituted from a frozen stock. Bacterial preparations rehydrated from room temperature may suffer from a lack of performance.

**EBPI recommends running duplicates for each sample. Each vial of reconstituted reagent can accommodate 1 full 96-well plate using the modified procedure above. ISO 21338 will require more than one vial for a plate. Plan accordingly**

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# LumoPlate™ Ultimate Matrix Kit

## Sample Preparation for testing

1) Water samples:

Adjust salinity of undiluted samples to 2 % using OAS (1243-115) by adding 1/10 total sample volume (eg. Add 500 µL OAS to 4.5 mL sample).

2) Solid samples:

Add 2.0 g of solid sample into 8.0 mL of Sample Diluent (1243-125) in a separate tube. Mix well for 5 minutes to homogenize the sample.

Adjust pH of all sample to 7.0 ± 0.2 if the sample pH is not between 6 and 8.5. Use 1.0 M NaOH or HCl solutions being sure not to increase the total volume of the sample by more than 5%.

If the oxygen content of the undiluted sample is less than 3 mg/L, oxygenate the sample by aeration or stirring

**DO NOT refreeze the reagent for use on subsequent days!**

## Prepare a dilution Series

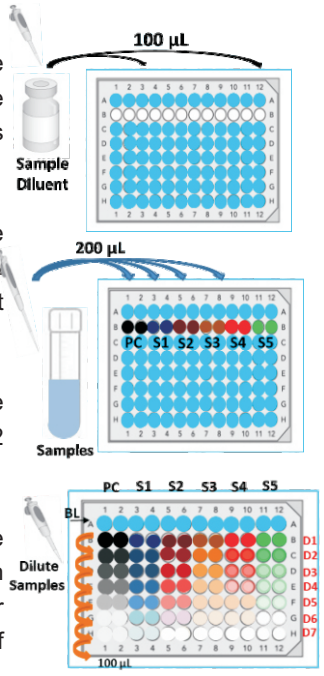
While the bacterial reagent is being reconstituted, prepare a sample dilution series according to ISO 21338 or directly on the microplate as described below. Include positive controls as necessary (See Validity Criteria). Equilibrate the plate to 15 °C prior to the first measurement and **ensure that the sample temperature is maintained at 15 °C throughout all measurements by storing plate on cooling block or in temperature controlled microplate luminometer**.

1. Between 5 and 10 dilution levels are required to calculate accurate $EC_{50}$. EBPI recommends diluting samples down the column and running duplicates for each sample. Using this format, 5 samples are run at 6 dilutions per plate, with controls.

2. Dispense 100 µL of Sample Diluent into all wells of the microplate **EXCEPT ROW B.** (**Row A** is used for non stressed bacteria readings and provide background levels of light production. **(calculation for correction factor, labelled BL**

3. Transfer 200 µL of each prepared sample stock to the appropriate wells in row B starting at column 3. Columns 1 and 2 are reserved for a positive control.

4. Prepare a dilution series down each column containing a sample by transferring 100 µL of sample from the first well in the column to the well below. Mix this new dilution. Repeat this transfer sequence for every well in the column. Discard 100 µL of sample from the wells in row H




**All wells should have a final volume of 100 µL. Cut a larger opening in pipette tips to more easily transfer samples with larger particulate.**

## Starting the Assay

1. Set the dispensing volume on the luminometer according to protocol followed (100 µL) and prime the dispenser with equilibrated *Allivibrio fischeri* reagent.

2. Load the microplate into the luminometer and start the run according to individual instrument protocols. Ensure the dispensing volume matches the volume of sample in each well (100 µL ).

**IMPORTANT: ISO 21338 requires different volumes of reagent and sample. The microplates included with this kit will ONLY accommodate EBPI's method. If larger plates are required, please contact EBPI for pricing. Sensitivity and accuracy of results is very similar using either method (see reference 2).**

3. Determine and record the maximum luminescence value of the test suspension during the first 5 s of contact. The maximum luminescence intensity is the peak-value, Ip. Transfer the plate to the incubator immediately after acquiring initial peak intensity. Store the cuvettes at (15 ± 1) °C during the incubation.

4. Determine and record the luminescence intensity after 5, 15 or 30 min as required

## Calculations and Data Interpretation

1. All calculations are performed with the software. If the sample has no colour or turbidity, the $EC_{50}$ values are calculated according to the ISO Standard 11348. If the sample has visible colour or turbidity, the operator must use the ISO 21338 standard to correct for these values.

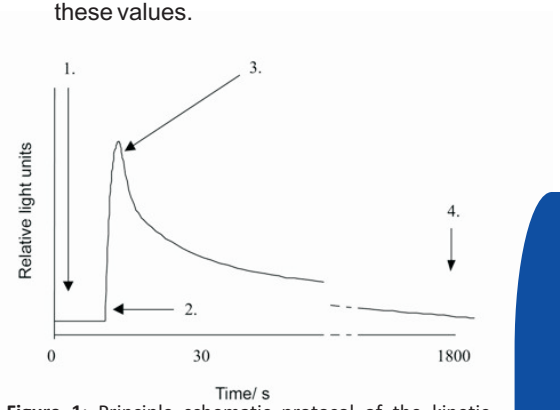



**Figure 1**: Principle schematic protocol of the kinetic toxicity determination. 1. Start measurement; 2. Inject bacteria; 3. Record peak value; Mix sample and record signal at 30 min.


**References:**

**1. ISO 11348-3, 2008 Determination of the Inhibitory Effect of Water Samples on the Light Emission of *Vibrio fisheri* (luminescent bacteria test)**

**2. Mortimer, M., Kasemets, K., Heinlaan, M., Kurvet, I., Kahru, A., (2008) High throughput kinetic *Vibrio fischeri* bioluminescence inhibition assay for study of toxic effects of nanoparticles. Tox. In Vitro 22, 1412-1417**


## Validity Criteria

Three test substances are commonly used to validate bacterial reagents and must cause between 20% - 80% light inhibition after 30 min incubation at the following concentrations :

3.4 mg/L 3,5-dichlorophenol

2.2 mg/L Zn(II), equivalent to 9.67 mg/L $ZnSO_4 \cdot 7H_2O$

18.7 mg/L Cr(VI), equivalent to 52.9 mg/L potassium dichromate