Application note
Triathler™ technical features

KINETIC TOXICITY DETECTION OF ENVIRONMENTAL SAMPLES

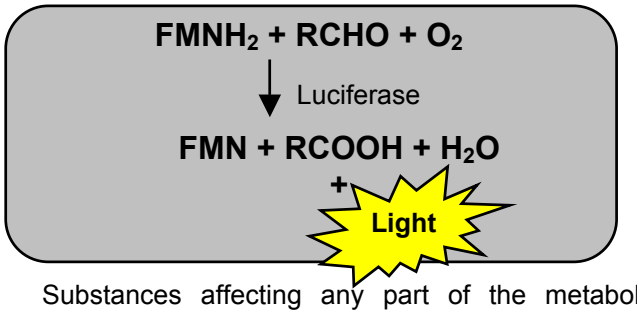
BioTox™ kit (Aboatox)

Introduction

Triathler™ luminometer equipped with automatic injector together with freeze-dried BioTox™ reagents provide fast and sensitive method for determination of the inhibitory effect of all water soluble samples, including suspensions of solid samples, on the light emission of *Vibrio fischeri* NRRL-B 11177. This application note describes the kinetic method for detection the toxicity, overruling the need for extra correction of quenching caused by color and turbidity.

Principles of the test

The BioTox™ reagent contains naturally luminescent *Vibrio fischeri* bacteria, producing luciferase as a part of its methabolic pathway.
Luciferase specifically reacts with $FMNH_2$ and catalyzes the following reaction.



Substances affecting any part of the metabolic pathway of the bacteria, directly affect to the amount of light emitted.

Assay Procedure on Triathler™

The inhibitions (INH%) caused by the sample dilutions to *V. fischeri* bacteria are determined and EC_{50} –values (sample concentration causing 50% inhibition) calculated using linear regression analysis. The sample dilutions and control sample (2% NaCl) are pipetted in the test tubes (500 µL each). The Triathler injector is filled with the *V. fischeri* reagent. The first tube containing the control sample (2% NaCl) is placed in Triathler and the measurement and injection of 500 µL of the reagent initiated simultaneously. The measurement is continued on kinetic mode for 5 seconds. The tube is placed in incubator at 15°C and the same procedure repeated for each sample. After incubation time of 15 minutes the end-point readings from the control and each sample are being measured.

Procedural Notes:

The tubes shall be shaken before the measurements in order to obtain homogenous suspension.
Any incubation time 5 – 30 minutes can be used instead of 15 minutes.

Results

Inhibitions (INH%) of each sample dilution are calculated according to the equations below and plotted on log-log scale. The EC_{50}-value is determined by using standard linear regression analysis.

$$KF = \frac{IC_{15}}{IC_0}$$

$$INH\% = 100 - \frac{IT_{15}}{KF \times IT_0} \times 100$$

Where:

KF = Correction factor.

IC_{15} = Luminescence intensity of the control after contact time (15 min) in CPS.

IC_0 = Maximum CPS value of the control during the 5 second kinetic measurement.

IT_{15} =Luminescence intensity of test sample after contact time (15 min) in CPS.

IT_0 = Maximum CPS value of the sample during the 5 second kinetic measurement.

Conclusions

The BioTox kit together with Triathler luminometer and automatic injector offer the fastest and most easy-to-use method for detection of toxicity of colorful and turbid samples, including water suspensions of solids (soil, waste and sediment). Also clear water samples can be measured.

Materials required

Triathler™ luminometer
BioTox™ kit (Code 1243-500)
Luminometer cuvettes or similar test tubes.
Incubator to maintain the reagent/samples at 15°C.

Product Information

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