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Sirius Tube Luminometer FB12/Sirius Software

Kinetic Luminescent bacteria test for Measuring the Toxicity of Solid and Coloured Samples with *Vibrio fischeri*

Measuring the toxicity of solid samples and coloured water samples has been time consuming, difficult and somewhat unreliable. Intense dark colour of the water samples and sedimentation of bacteria, turbidity and colour of the solid samples causes problems. The Flash Method is designed to overcome these apparent problems, and more: the Flash Method is extremely fast, with some of the samples the result is obtained within 30 seconds.

The Flash method is based on the BioTox™ test utilizing bioluminescent *Vibrio fischeri* bacteria. The effect of colour and turbidity is automatically corrected for each sample, and no complicated estimations are needed. The measurement is performed in a kinetic mode with an instrument capable to dispense the reagents and measure at the same time.

The measurement starts by dispensing the *Vibrio fischeri* reagent to the sample, causing a rapid increase of luminescence. The maximum value of luminescence (the peak) is reached normally within 1-2 seconds. The peak height is inversely proportional to the colour and turbidity of the sample. The second value (the end point) for the toxicity calculation is measured after 15 or 30 minutes. The toxicity of the sample is calculated as the relationship of the end point value and the peak value. The result is corrected with the correction factor obtained from the non-toxic reference sample (2% NaCl solution).

Materials

Reagents :	<i>Vibrio fischeri</i> Reagent Reagent Diluent Sample Diluent (20% NaCl) 0,1M NaOH and HCl for pH adjustment
Luminometer:	Sirius Tube Luminometer with 1 dispenser
Software:	FB12 / Sirius PC Software
Incubator:	15°C
Other Materials:	Luminometer cuvettes 75 mm x 12 mm Ø normal laboratory equipment: balance, mixer, pH-meter, pipettes with wide tips, tubes, etc.

FB12/Sirius PC Software Protocol settings

Sirius Luminometer with 1 injector was used to perform a kinetic measurement after reagent injection.
Single Kinetic protocol for the peak determination :

Delay time 1,0 s
Total duration 5,0 s
Sampling time 1,0 s
No. of data points 25
Interval time 0,2 s
Start timer by Door close
Number of replicates 1

Quick Measurement protocol for end point measurements:

Delay time 1,0 s
Measurement time 0,2 s

Procedures

Reconstitution of the Reagents

Immediately after taking the *Vibrio fischeri* reagent out of the freezer, add the content of cold ($3^{\circ}\text{C} \pm 3^{\circ}\text{C}$) Reagent Diluent in the vial of the *Vibrio fischeri* reagent. The reconstituted reagent should be equilibrated at $+4^{\circ}\text{C}$ for at least 30 minutes. Take the bacterial suspension at measuring temperature (15°C) approximately 1 hour before starting the measurements. The reconstituted reagent must be used within the same day and it cannot be stored. The content of 1 vial is sufficient for approximately 35 measurements.

Sample Preparation

- 1) Prepare
 - Solid samples:
Stir 2,0 g sample into 8,0 ml of 2% NaCl (1/10 dilution of Sample Diluent) in separate tubes and mix for 5 minutes.
 - Water samples:
Adjust the salinity of the sample by adding 1 ml of 20% NaCl solution to 9 ml of the sample and mix gently.
- 2) Measure the pH and adjust to $7 \pm 0,2$ if not between 6 – 8,5.
- 3) Dilute each sample with 2% NaCl to obtain proper dilution series (for example 1:2, 1:4, 1:8 and 1:16).
- 4) Use the 2% NaCl-solution as control sample

Note! If the toxicity level of the sample is totally unknown, screening/range finding test is suggested to be performed by using for example undiluted, 1/10 and 1/100 diluted samples.

Bioluminescent Measurement

1. Mix the samples and sample dilutions well (including the control sample) and pipette 300 μl of each sample into the luminometer cuvette. Incubate at 15°C at least 10 min prior to testing.
2. Set the dispensing volume to 300 μl and prime the Dispenser with the *Vibrio fischeri* Reagent.
3. Load the first sample into the instrument and start the run using Single Kinetics protocol (The bacteria is dispensed to the sample and the luminescence is followed in kinetic mode for 5 seconds). Transfer the sample to the incubator at 15°C and repeat this step for all the samples with fixed time intervals. Obtain S_{max} .
4. Measure second value of luminescence after 15 and/or 30 minutes immediately after mixing. Use the Quick Measurement protocol. Obtain $S_{15\text{min}}$ and/or $S_{30\text{min}}$.

Calculation and Interpretation of the results

Calculation of the inhibitions and EC_{50} values are performed according to the ISO standard. The only exception is that S_{max} is used as the initial luminescent value instead of the initial luminescence value of pure bacterial suspension.

The toxicity of the sample is evaluated by calculating the ratios $S_{15\text{min}}/S_{\text{max}}$ and $S_{30\text{min}}/S_{\text{max}}$.

If the S/S_{max} ratio is less than 0.8 the sample is normally considered toxic. These S/S_{max} ratios can be plotted against sample concentration. The EC_{20} and EC_{50} values (= concentrations that inhibit the luminescence by 20% and 50%) can be defined from the plot.

Note! If the peak height of the sample is less than 10% of the control, the sample must be diluted further.

Example

Figure 1 shows the principle of the kinetic measurement. Figure 2 shows the difference between toxic and non-toxic samples in the kinetics of the light emission. Curve A represents the normal behaviour, where there is no observable toxicity or absorption. Curve B represents highly toxic sample without absorption effect and curve C includes both toxic and absorption effect. A very strong absorption effect without toxic action is visible in the curve D.

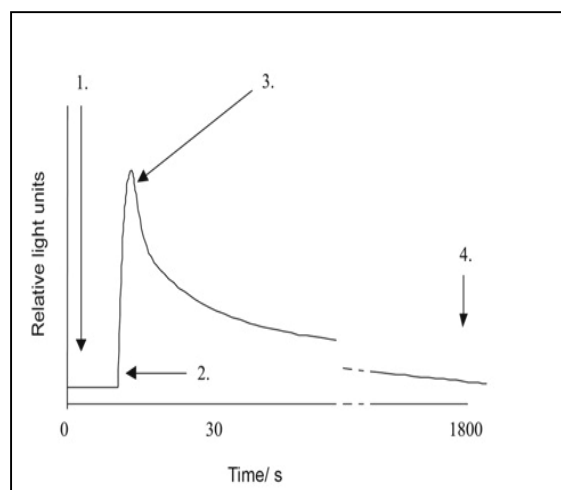


Figure 1. Principal schematic protocol of the kinetic toxicity determination. Key: 1. Start measurement; 2. Inject bacteria; 3. Record peak value from 0 s to 5 s; Mix sample and record signal at 30 min.

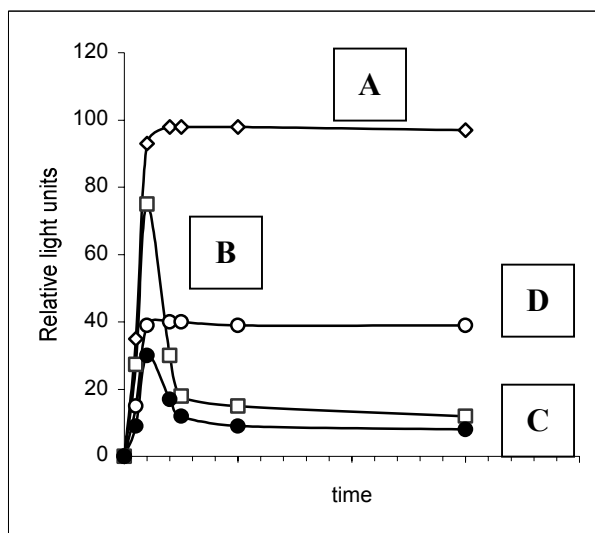


Figure 2. Typical kinetic behaviour of toxic and non-toxic samples. Key: A: clear, non toxic sample; B: clear, toxic sample; C: turbid/colourful, toxic; D: turbid/colourful, non-toxic.

Acknowledgement

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