

Luminescent Detection of Detergent Residuals

Application Note 201

Introduction

Luminescent ATP measurement is widely used for hygiene monitoring in industry. The measurement is mainly used for testing of cleaning efficiency by detecting residual ATP from recently washed surfaces. Therefore some traces of detergents can be present when samples are collected. These traces can interfere with the measurement by inhibiting the ATP measurement and resulting in false negative results.

The reliability of the results is extremely important in any testing performed in industry. In addition, the residues of eg. tensides due insufficient rinsing cause chemical risk in food processing. Therefore, we have developed a completely new application for the detection of the cleaning agents and disinfectants. These detergents can be detected easily and at very low concentrations with the luminescent BioTox™ Toxicity Screening System. The method can be applied to both rinse waters and process surfaces.

Reagents and Equipment

Reagents

- 1243-500 BioTox™ kit

The Kit is used according to the kit insert with the exceptions mentioned in the sections Reconstitution of the Reagent and Bioluminescence Measurement.

Instrumentation

- Lumitester™ PD-10 or C-100 luminometer
- 15°C cooling block for incubation of the *V.fischeri* reagent and the samples

Other Materials

- luminometer cuvettes
- 500 µl pipette
- pipette tips
- sample tubes

Procedures

Test principle

The test is performed by combining equal amounts of sample and *Vibrio fischeri* bacterial reagent in a luminometer cuvette at 15°C. The luminescence is measured after a 5 min. contact time. The luminescence of each sample is compared to the luminescence of the control sample. The relative inhibition (INH%) to the luminescence activity caused by the sample reveals the detergents and/or disinfectants present in the sample.

Reconstitution of the Reagent

Reconstitute the *Vibrio fischeri* reagent by adding the contents of one vial of +4°C Reagent Diluent. The reconstituted reagent should be equilibrated at +4°C for at least 30 minutes. Then stabilise the reagent at measuring temperature (15°C) for appr. 1 h before pipetting into the cuvettes.

Sample Preparation

Collect all samples into chemically inert containers and stabilise to measuring temperature. The samples cannot be stored by adding any chemicals and they should be analysed as soon as possible after sample collection.

A. Control sample

Take at least 2 ml sample of tap water used for rinsing. This sample is used as the control sample.

B. Wet process surfaces

If the surface is wet, rub the surface area (about 6 x 6 cm²) with the pipette tip for about 20 s and take carefully with the pipette at least 1,5 ml sample to a suitable tube.

C. Dry process surfaces

Pipette 2.0 ml of rinse water to the surface to be tested. Rub the surface area (about 6 x 6 cm²) with the pipette tip for about 20 s. Remove at least 1,5 ml of the liquid to a suitable tube for the measurement.

Bioluminescence Measurement

Note! The measurements are preferred to be performed at 15°C.

Pipette 500 µl of bacterial test suspension to sufficient number of luminometer cuvettes. The series must contain always a rinse water sample as control and the test should be made with at least two replicates. Total number of cuvettes needed is 2 x number of samples + 2 controls. Stabilise the cuvettes at measuring temperature for at least 10 min.

1. Add 500 µl of each sample into the cuvette containing the bacterial test suspension. Start with 500 µl of the clean water to obtain the control sample.
2. Incubate the cuvettes exactly 5 min. Measure the luminescence activity with the luminometer.

Procedural Notes

Detergents containing volatile chemicals (for example hydrogen peroxide) may result in reduced inhibition when tested with this method because of the strong evaporation.

An organic contamination of the sample by readily biodegradable nutrients (for example sugars, organic nitrogen, etc.) may cause induction in the light emission.

Calculations

The inhibition efficiency of the sample is calculated to each sample as follows:

$$\text{INH}\% = 100 - \frac{100 \times \text{IT}_t}{\text{IC}_t}$$

Where:

INH% = Inhibition efficiency

IT_t = Luminescence activity of the sample after the contact time t.

IC_t = Luminescence activity of the control after the contact time t.

The inhibition efficiency given as a result is calculated as an average of the two measurements.

Interpretation of the results

If the inhibition caused by the sample is below 20 %, the amount of residuals is less than the detection limit for the detergents/disinfectants used. If the inhibition is between 20 - 50 %, the sample contains low amounts of residuals and if over 50 % inhibition is achieved, the sample contains high amount of residuals. The rinsing of the surfaces should be repeated until below 20% inhibition level is achieved.

The exact amount of residuals can be determined by comparing the inhibition efficiency of the sample to a pre-determined inhibition curve for the specific detergent used.

References

Lappalainen, J., Loikkanen, S., Havana, M., Karp, M., Sjöberg, A.-M. and Wirtanen, G. (2000). Microbial testing methods for detection of residual cleaning agents and disinfectants – Prevention of ATP bioluminescence measurement errors in the food industry. *J. Food Prot.* 63, pp. 210 – 215.

Ordering Information

- 1243-500 BioTox™ Kit for 144 determinations
- PD-10 Lumitester™ PD-10 luminometer
- C-100 Lumitester™ C-100 luminometer
- 260009-26 Cooling Block, 15 °C
- PD-10c Cuvettes for PD-10, 30 pcs
- 2174086c Cuvettes for C-100, 100 pcs

