CheckLite 250 Plus

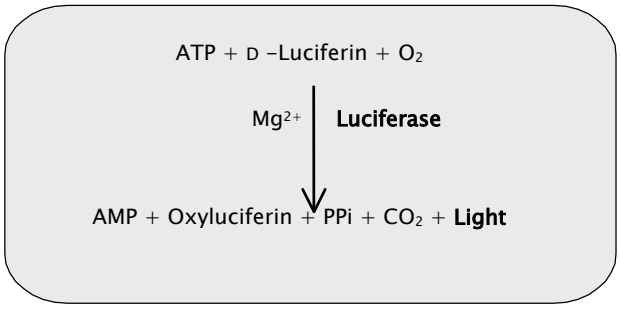
Kit for microbial biomass assay and hygiene monitoring for 250 assays
ID-No. 1 002 648
User's Manual

CheckLite 250 Plus is a kit for microbial biomass assay and hygiene monitoring based on the ATP measuring method. It contains thermostable firefly luciferase.

CheckLite 250 Plus is easy to use and performs rapidly in microbial biomass assays.

Principle of Measurement

The bioluminescence reagent contains firefly luciferin and luciferase. Luciferase reacts with ATP and is the catalyst of the following reaction:



The amount of bioluminescence produced in the reaction is in direct proportion to the amount of ATP in the sample.

All living cells, including microorganisms, have ATP as their energy source. Therefore, the total cell mass can be determined by measuring bioluminescence in the luciferase reaction after extracting ATP from the cells using the ATP releasing reagent in this kit.

Storage of Kit

Store the kit at 2-8°C in a refrigerator. **DO NOT FREEZE.**

Kit Contents

1. Luciferin-luciferase reagent:
   5 green-labeled vials
   These vials contain purified firefly luciferase, D-luciferin, magnesium salt, TRICINE, BSA and DTT in lyophilized form.
2. Solvent water:
   5.5 ml x 5 pink-labeled vials
   These vials contain ultra-pure water for dissolving the luciferin-luciferase reagent.
3. ATP releasing reagent:
   11 ml x 2 light blue-labeled vials
   These vials contain a surfactant used for extracting ATP from microbial cells.

Preparing reagents

1. Bioluminescence reagent

(1) Luciferin-luciferase reagent is kept under vacuum in a green-labeled vial.

(2) Pour the solvent water from the pink-labeled vial into the opened green-labeled vial and leave it at room temperature for a few minutes.

(3) Stir the vial gently so as not to produce foam until the contents are completely dissolved.

(4) Do not touch the rim of the vial or the top of the rubber plug directly since this may raise the blank value of the reagent under certain circumstances.

(5) One vial of luciferin-luciferase reagent can be used for more than 50 assays under regular conditions.

Instructions for Use

1. Handling samples

The concentration of ATP in living cells changes rapidly. Therefore, extract ATP from cells or freeze the cells immediately after sampling. Even in the case of extraction, the inhibition or destruction of ATP-degrading enzymes may be necessary to prevent enzymes from decreasing the concentration of ATP.

2. Pretreating samples

(1) Solid samples:
Treat the sample with a stomacher or a homogenizer and then use the supernatant for the following measurement.

(2) Liquid samples:
When the sample solution is turbid, colored, or contains inhibitory ions, such as Cl^-, dilute the sample as necessary.

3. Measurement

(1) Pour 100 µl of the sample that has been handled as described above into a test tube for measurement, and add 100 µl of the ATP releasing reagent solution to the test tube.

(2) Leave it at room temperature for 10–60 seconds to extract ATP from the microbial cells. The time required for extraction varies according to the species of the microorganisms. For example, it takes 10–20 seconds to extract ATP from bacterial cells and 60 seconds are needed for yeast cells.

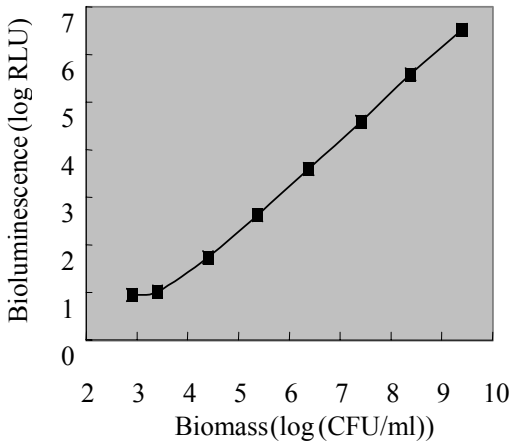
(3) Immediately after extraction, add 100 µl of the luciferin–luciferase reagent solution and measure the amount of bioluminescence with a luminometer, such as Lumitester C–100 (ID No. 1 002 647).

(4) In order to plot a calibration curve, measure the amount of bioluminescence from incremental dilutions of ATP standard solution using the method described above.

(5) The number of microbial cells (Colony Forming Units, CFU) can be obtained based on the correlation between the amount of ATP and the number of CFU counted in advance using to the traditional colony counting method.

An example of assaying *E. coli* is described below.

1. Cultivated *E. coli* is diluted appropriately.
2. Transfer 100 µl of sample to a test tube.
3. 100 µl of ATP releasing reagent is added to the tube.
4. After 10 seconds, 100 µl of luciferin–luciferase solution is added.
5. The amount of bioluminescence (RLU; Relative Light Units) is measured using a luminometer.
6. CFU is counted on an agar plate.
7. A typical calibration curve is shown below.



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