kikkoman

Kit for microbial biomass assays

CheckLite
ATP Eliminating Kit

For 250 assays
Code 61306

. User's manual

CheckLite ATP Eliminating Kit is used for microbial biomass assays based on the ATP measuring method. It contains ATP degrading enzymes developed by Kikkoman Corporation.

CheckLite ATP Eliminating Kit is characterized by its ease of use and rapid performance in highly sensitive microbial biomass assays.

Principle of Measurement

The amount of bioluminescence produced by firefly luciferin and luciferase is in direct proportion to the amount of the ATP present 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 with the luciferase reaction, after extracting ATP from the cells using the ATP releasing reagent in CheckLite 250 Plus (Kikkoman Biochemifa Company, Code 61305) provided that extracellular ATP is low.

However, a major problem is that extracellular ATP can be several magnitudes higher in some samples, which obstructs the sensitive detection of intracellular ATP. Kikkoman ATP Eliminating reagent eliminates extracellular ATP to very low concentrations compared with a previous method and enables the sensitive detection of bacteria.

Storage

Store the kit at 2-10°C in a refrigerator. Do not freeze.

Composition of Kit

1. **ATP eliminating reagent;**
   5 vials reddish pink-labeled
   These contain ATPase, adenosine phosphate deaminase, MES and BSA.
2. **Reconstitution buffer for ATP eliminating reagent;**
   5 vials yellow labeled
   These contain ultra-pure water for dissolving the ATP eliminating reagent.

Preparation of reagents

(1) ATP eliminating reagent is kept under vacuum in a red-labeled vial.
(2) Pour the Reconstitution buffer for ATP eliminating reagent from the yellow-labeled vial into the opened red-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 with your hands, since this will sometimes raise the blank value of the reagent.
(5) One vial of ATP eliminating reagent can be used for more than 50 assays under normal condition.

Direction for Using the Kit

1. Notes for 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 the ATP.

2. Pretreatment of 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. ATP degradation of samples

(1) Put 1 ml of sample into the test tube.
(2) Add 0.1 ml of ATP eliminating reagent into the test tube and mix well.
(3) Let the test tube stand at room temperature for 30 to 40 minutes. It is important to keep the reaction time constant to obtain good reproducibility. If testing multiple samples, each test tube should stand at room temperature for the same time.
(4) After reaction at room temperature above, the samples are used for the bioluminescence assay below.

4. Example of assaying ATP

(1) Place 100 µl of the sample treated as described above into a test tube for measurement, and add 100 µl of the ATP releasing into the test tube.
(2) Leave it at room temperature for 10-60 seconds to extract ATP from the microbial cells. The time required for the 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 in the case of yeast cells.
(3) Immediately after the extraction, add 100 µl of the luciferin-luciferase reagent and measure the amount of bioluminescence with a luminometer such as a Lumitester C-110 (Kikkoman Biochemifa Company, Code 61911).
(4) In order to make a calibration curve, measure the amount of bioluminescence from stepwise dilutions of ATP standard solution according to the method described above.
(5) The number of microbial cells (Colony Forming Units, CFU) can be estimated based on the correlation between the amount of ATP and the number of CFU counted beforehand according to the traditional colony counting method.

Data treatment

Plotting bioluminescence count (RLU, Relative Light Units) and the number of microbial cells (Colony Forming Units, CFU) on a double log graph, the relationship between RLU and CFU can be obtained. Once the relationship between RLU and CFU in some control samples has been obtained, CFU can be estimated directly from the RLU by using the resulting calibration curve.

Produced by

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