Kit for highly sensitive microbial biomass assays

CheckLite HS Set

For 100 assays
Code 61310

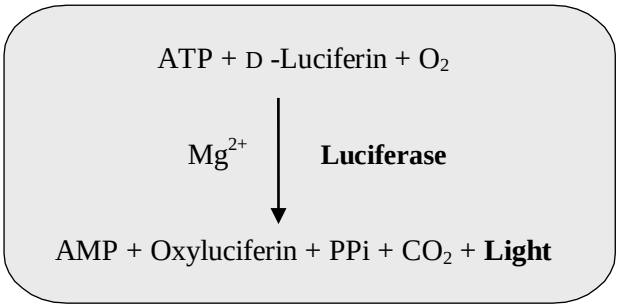
User's manual

CheckLite HS Set is a kit for microbial biomass assay based on the ATP measuring method. It contains thermostable firefly luciferase and ATP degrading enzymes developed by Kikkoman Corp.

CheckLite HS Set is characterized by its ease of use, rapid performance and high sensitivity in microbial biomass assays.

Principle of Measurement

The bioluminescence reagent contains firefly luciferin and luciferase. Luciferase specifically reacts with ATP and catalyzes the following reaction.



The amount of bioluminescence produced in the reaction above is in direct proportion to the amount of the 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 the bioluminescence with 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.

Composition of Kit

1. Luciferin-luciferase reagent HS:
   (2 vials deep green-labeled)
   These contain purified firefly luciferase, D-luciferin, magnesium salt, TRICINE, BSA and DTT in lyophilized form.
2. Reconstitution buffer for luciferin-luciferase reagent:
   (5.5 ml x 2 vials pink-labeled)
   These contain TRICINE buffer for dissolving the luciferin-luciferase reagent.
3. ATP releasing reagent:
   (5.5 ml x 2 vials light blue-labeled)
   These contain a surfactant used for extracting ATP from microbial cells.
4. ATP eliminating reagent;
   (2 vials reddish pink-labeled)
   These contain ATPase, adenosine phosphate deaminase, MES and BSA.
5. Reconstitution buffer for ATP eliminating reagent;
   (5.5 ml x 2 vials yellow labeled)
   These contain MES buffer for dissolving the ATP eliminating reagent.

Preparation of reagent

Bioluminescence reagent

(1) Luciferin-luciferase reagent is kept under vacuum in a deep green-labeled vial.
(2) Pour the reconstitution buffer from the pink-labeled vial into the opened deep green-labeled vial and leave it at room temperature for a few minutes. (A slight sediment may form in the reconstitution buffer during storage. This will not interfere with test results.)
(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 luciferin-luciferase reagent can be used for more than 50 assays under normal condition.

ATP eliminating reagent

(1) ATP eliminating reagent is kept under vacuum in a reddish pink-labeled vial.
(2) Pour the reconstitution buffer from the yellow-labeled vial into the opened reddish pink-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 the 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.

3. Measurement

(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 reagent solution 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.

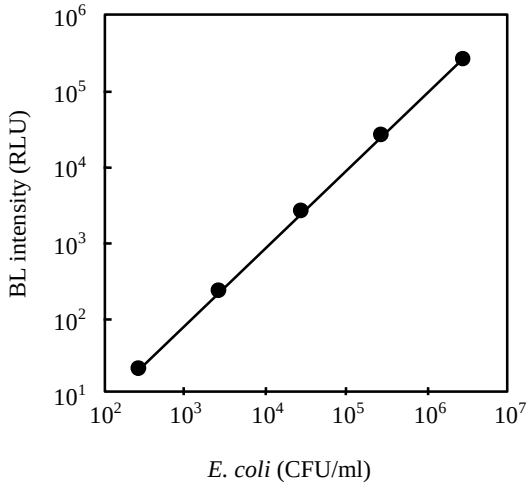
(3) Immediately after the extraction, add 100 µl of the luciferin-luciferase reagent solution and measure the amount of bioluminescence with a luminometer such as a Lumitester C-110 (Kikkoman Biochemifa Company, Code 61911).

(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 obtained based on the correlation between the amount of ATP and the number of CFU counted beforehand according to the traditional colony counting method.

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

1. Cultivated *E. coli* is diluted appropriately and the extracellular ATP is eliminated as described above.
2. Transfer 100 µl of sample into a test tube.
3. 100 µl of ATP releasing regent 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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