kikkoman

Kit for microbial biomass assay

CheckLite AT100

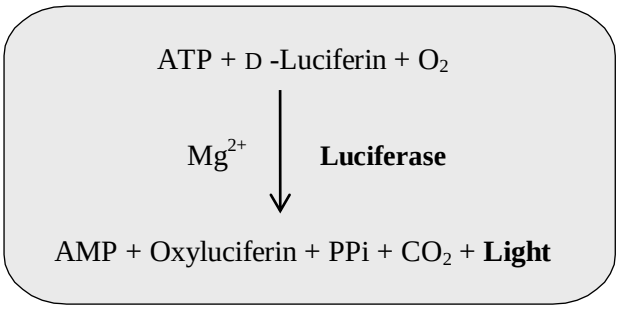
For 100 assays
(Code 61312)

User's manual

CheckLite AT100 is a kit for microbial biomass assay based on the ATP measuring method, especially suitable for aseptic test of low acidic beverages and retort foods after appropriate incubation. It contains thermostable firefly luciferase and an ATP eliminating reagent. CheckLite AT100 is characterized by its ease of use and rapid performance 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 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.

The ATP levels due to extracellular ATP of emulsive products, such as milk, cream, and products containing milk can be several orders of magnitude higher than the background ATP levels found in other food products. This high level of ATP interferes with the sensitive detection of intracellular ATP from contaminating microbes. With Kikkoman's CheckLite AT100, somatic cells and micelle structure are ruptured and the released ATP and any extracellular ATP are degraded to very low concentrations compared with other ATP methods, enabling the sensitive detection of bacteria in emulsive product samples.

Storage of Kit

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

Composition of Kit

A. 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.

B. Reconstitution buffer for Luciferin-luciferase reagent:
5.5 ml x 2 vials pale pink-labeled
These are Tricine buffer for dissolving the luciferin-luciferase reagent.

C. ATP releasing reagent:
5.5 ml x 2 plastic vials light blue-labeled
These contain a surfactant used for extracting ATP from microbial cells.

D. ATP eliminating reagent:
2 vials reddish pink-labeled
These contain enzymes for ATP degradation and buffer salts in lyophilized form.

E. Reconstitution buffer for ATP eliminating reagent:
5.5 ml x 2 vials yellow-labeled
These contain sterilized water for dissolving the ATP eliminating reagent.

F. Sample dilution buffer:
25 ml x 4 plastic bottles dark blue-labeled
These are MES buffer containing sucrose used for diluting of assay samples.

G. Sample treating reagent:
5.5 ml x 2 plastic vials brown-labeled
These contain a detergent used for releasing ATP from somatic cells and for degrading micelles of milk fat.

Precautions

In order to get fully the performance of this product, please carefully read the following points.

(1) Do not use an expired kit. (Expiration date is printed on the kit box)
(2) Recommended luminometers should be used to measure luminescence intensities.

(3) Take out the kit from the refrigerator (2-8°C). Allow the reagents to stand for approximately 20 minutes at room temperature (20-30°C) before use.
(4) The luminescence reagent HS is plugged under vacuum after freeze-drying. Since there is a possibility that air may enter rapidly and content may disperse if a rubber plug is opened at a dash, please remove the rubber plug in accordance with the method of preparing a reagent.
(5) A slight sediment may form in the reconstitution buffer for Luciferin-luciferase reagent during storage. This will not interfere with test results.
(6) Static electricity on LumiTube or Lumitester may cause abnormal values on Lumitester. To prevent static electricity, wipe the LumiTube and the Luminometer with a moistened towel.
(7) During operation, use laboratory gloves and a mask to avoid the contamination of ATP or microbes.
(8) Please don't mix the reagent of a different lot (it indicates on a reagent bottle label).
(9) An outlying observation may be shown by Lumitester, if an electrical noise is generated by an electrical apparatus, such as a microwave oven and a mixer, etc. Please use the Lumitester after confirmation of that there is no apparatus which generate an electrical noise over surrounding area.
(10) Commercial sterilized micropipette tips or autoclaved micropipette tips, which were set in the racks by hands wearing laboratory gloves, should be used.
(11) Be careful not to touch at the mouth of the reagent bottles and vials, or the inner surface of a cap when using this product.
(12) Please measure light intensity immediately after addition and sufficient agitation of the luminescence reagent.
(13) The time required for extraction is normally within 20 sec. However it varies with kinds of microbe in some case. In order to perform measurement properly, please use a definite period of time after determination of the optimal time.
(14) Although this kit is easy for presuming a microbial biomass quickly, the exact number of microorganisms may be unable to be determined. If you need the exact number of microorganisms, please measure the number of microorganisms based on an officially approved technique.
(15) Please measure a sample after certainly checking that the reagents of the kit are not contaminated by ATP or the microbe. The test method of checking contamination is described in the section one of the "Direction for Using the Kit".
(16) The standard curve used for performing microbial biomass assay should be prepared under same operating conditions, such as a kind of microbe and composition of the culture medium or sample to be used. In case of preparing ATP standard curve, the ATP eliminating reagent should be replaced by ATP free distilled water.

Preparation of reagents

1. Bioluminescence reagent solution (A')

(1) Luciferin-luciferase reagent HS (A) is kept under vacuum in a deep green-labeled vial.
(2) Pour the reconstitution buffer from the pale pink-labeled vial (B) into the opened deep green-labeled vial (A) 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 luciferin-luciferase reagent HS can be used for more than 50 assays under normal conditions.

2. ATP eliminating reagent solution (D')

(1) ATP eliminating reagent (D) is kept under vacuum in a reddish pink-labeled vial.
(2) Pour the reconstitution buffer from the yellow-labeled vial (E) into the opened reddish pink-labeled vial (D) 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) One vial of ATP eliminating reagent can be used for more than 50 assays under normal condition.

3. Other reagents (C, F, G)

(1) Bring these reagents to room temperature.
(2) Stir the vials gently. Do not shake them vigorously.

Storage of the reagents after preparation

(1) **Bioluminescence reagent solution (A')**
Store below -4°C in a freezer.
It can be stored at 2-8°C temporally when used several times a day.

(2) **ATP releasing regent (C)**
Store below 2-8°C in a refrigerator.

(3) **ATP eliminating reagent solution (D')**
Store below -4°C in a freezer.

(4) **Sample dilution buffer (F)**
Store below -4°C in a freezer.

(5) **Sample treating reagent (G)**
Store below 2-8°C in a refrigerator.

Instrumentation besides the kit

(1) **Luminometer**
Photon counting-type luminometer such as a Lumitester C-110 (Kikkoman Biochemifa Company, Code 61910) is necessary.

(2) **Test tubes**
Polystyrene test tubes (12 mm i.d. x 55 mm) are needed for sample treatment reaction and for measurement.

(3) **Pipetters and sterilized pipette tips**
Devices that can measure 100 µl, 700 µl, or 800 µl are required.

Direction for Using the Kit

1. Checking contamination of the reagents

Check whether the reagents are contaminated with ATP or microorganisms, when the reagent is used again after storage.

When reagents are contaminated, a large amount of luminescence is detected only with the reagents, and an accurate assay result cannot be obtained.

Use the following reagents to obtain a blank value.

Sample dilution buffer (F)	100 µl
+ATP releasing reagent (C)	100 µl
+Bioluminescence reagent solution (A')	100 µl

Measure the blank value of the reagents before performing any assays. A large amount of luminescence will be obtained when the reagents are contaminated

When a large amount of luminescence is obtained, replace the reagents with fresh ones and repeat same operation as mentioned above.

2. Measurement

Method A (for low acidic beverages, etc.)

(1) Place 800 µl of the sample dilution buffer (F) into a test tube, and add 100 µl of a sample and 100 µl of the ATP eliminating reagent solution (D') into the test tube.

(2) Stir the test tube to mix them well and transfer a 100 µl aliquot into another test tube for measurement. Allow the sample to stand at room temperature for 30 minutes to eliminate any ATP from anything other than microbial cells.

(3) Add 100 µl of the ATP releasing reagent (C) into the test tube and stir to extract ATP from the microbial cells.

(4) Add 100 µl of the bioluminescence reagent solution (A') and immediately measure the amount of bioluminescence with a luminometer such as a Lumitester C-110.

Method B (for raw milk, milk rich products, and retort foods, etc.)

(1) Place 100 µl of the sample treating reagent (G) into a test tube, and add 100 µl of a sample into the test tube. Stir the test tube to mix them well.

(2) Add 700 µl of the sample dilution buffer (F) and 100 µl of the ATP eliminating reagent solution (D') into the test tube.

(3) Stir the test tube to mix well and transfer a 100 µl aliquot into another test tube for measurement. Allow the sample to stand at room temperature for 30 minutes to eliminate ATP from anything other than microbial cells.

(4) Add 100 µl of the ATP releasing reagent (C) into the test tube and stir to extract ATP from the microbial cells.

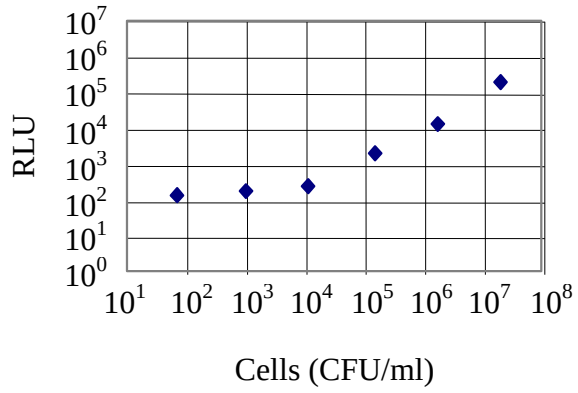
(5) Add 100 µl of the bioluminescence reagent solution (A') and immediately measure the amount of bioluminescence with a luminometer.

Examples of standard curves are shown below.

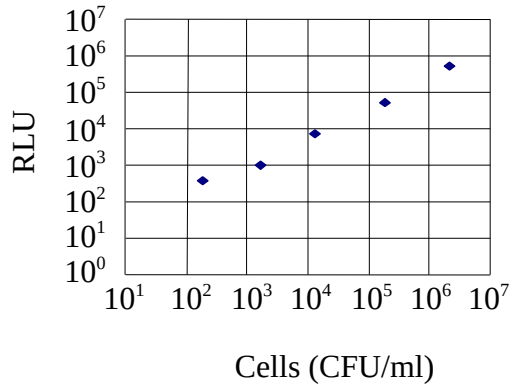
The number of microbial cells (Colony Forming Units, CFU) can be obtained based on the correlation between the amount of ATP and the number of CFU counted beforehand according to a traditional colony counting method.

Pseudomonas

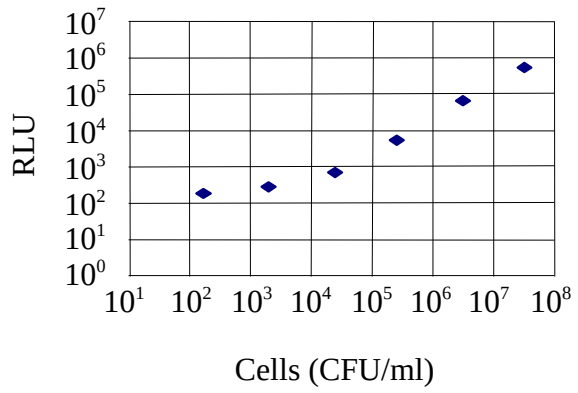
<Non-dairy creamer (vegetable oil)>



***Bacillus* <Cream>**



***Klebsiella* <Creamer (milk)>**



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