Luminescent Mycoplasma Detection Kit

Cat #: orb3148269 (manual)

Product Features

Product name: Luminescent Mycoplasma Detection Kit

Catalog number: orb3148269

Sample Types: Cells

Storage: Stored at -20℃ for 12 months, protected from light

Assay Principle

The Luminescent Mycoplasma Detection Kit is based on the activity of Mycoplasma specific kinase and is detected through the luminescence reaction catalyzed by ATP-dependent luciflucinase. By comparing the changes in ATP content before and after the addition of detection reagents, it determines whether the sample is contaminated with Mycoplasma. After Mycoplasma is lysed, it releases a specific kinase that reacts with the substrate and catalyzes the conversion of ADP to ATP. The whole experimental operation consists of two steps: the first step is to add Mycoplasma Reagent A to the sample to detect the original ATP content; the second step is to add Mycoplasma Reagent B, if the sample is contaminated by Mycoplasma, its unique kinase can catalyze the conversion of ADP to ATP, at this time, it is the sum of the original ATP content and the newly generated ATP content catalyzed by the specific enzyme of Mycoplasma. By calculating the ratio of luminescence reading value B to A, it is possible to determine whether there is Mycoplasma contamination. If the ratio is greater than 1.2, it indicates Mycoplasma contamination, the higher the ratio, the higher the degree of contamination. If the ratio is less than 0.9, it indicates no Mycoplasma contamination. If the ratio is between 0.9 and 1.2, it is recommended that the cells (including the original culture medium) continue to be cultured for 24-48 h before retesting.

Kit Components

Kit components	Size		Storage conditions
	20 T	200 T	
Mycoplasma Assay Buffer	1mL	10 mL	-20℃, protect from light
Luciferase	1 μL	10 μL	-20℃
Mycoplasma Substrate A	1	1	-20℃, protect from light
Mycoplasma Reagent B	1 mL	10 mL	-20℃
Postive Control	200 μL	1 mL	-20℃

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Materials Required but Not Supplied

- Sterile tubes, precision pipettes, disposable pipette tips
- Centrifuge, 96-well black plate or 96-well white plate
- Sterilized water, PBS or fresh culture medium
- Luminometer or multimode reader

Reagent Preparation

Mycoplasma Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Stored at -20℃, protected from light.

Luciferase: Ready to use as supplied. Stored at -20℃.

Mycoplasma Reagent A: Prepare before use, dissolve the Mycoplasma Substrate A with the Mycoplasma Assay Buffer, then transfer the dissolved Mycoplasma Substrate A and Luciferase to the bottle containing Mycoplasma Assay Buffer, mix well to obtain Mycoplasma Reagent A. Equilibrate to room temperature before use. Pack according to usage requirements and stored at -20℃, protected from light.

Mycoplasma Reagent B: Ready to use as supplied. Equilibrate to room temperature before use. Pack according to usage requirements and stored at -20℃.

Positive Control: Ready to use as supplied. Equilibrate to room temperature before use. Pack according to usage requirements and stored at -20℃.

Note: All reagents need to avoid repeated freezing and thawing, it is recommended to pack, containers used for packaging must not have ATP contamination.

Sample Preparation

1. Adherent cells: Take 0.2-1 mL of cell culture supernatant before cell digestion, centrifuge at 1,500 rpm for 5 min, and take the supernatant for detection.

2. Suspended cells: Take 0.2-1 mL of cell suspension during cell passage, centrifuge at 1,500 rpm for 5 min, and take the supernatant for detection.

3. Resuscitated cells: Add frozen cells to fresh complete culture medium after thawing, cultured for 1-2 h, take 0.2-1 mL of cell culture medium, centrifuge at 1,500 rpm for 5 min, and take the supernatant for detection.

Note:

1. After cell passage or digestion, the detection signal of Mycoplasma will be reduced. If samples are taken for testing after cell passage or digestion, Samples should be taken 24 h after passage or digestion.

2. It is best to test the sample immediately after collection, or it can be placed at 4℃ for testing on the same day or stored at -80℃ for testing within 6 months. Samples stored at low temperatures need to equilibrate to room temperature before they can be used for testing.

Assay Procedure

1. Add 50 µL of Samples, Positive Control and Negative Control (such as sterilized water, PBS and fresh medium) to the 96-well black plate or 96-well white plate, respectively.

2. Add 50 µL of Mycoplasma Reagent A to each well, mix well and incubate at room temperature for 5 min, then measure the luminescence reading value RLU_A with multimode reader.

3. Add 50 µL of Mycoplasma Reagent B to each well, mix well and incubate at room temperature for 10 min, then measure the luminescence reading value RLU_B with multimode reader.

4. Calculate Ratio =RLU_B/RLU_A. Referring to Table 1, if the ratio is greater than 1.2, it indicates Mycoplasma contamination; if the ratio is less than 0.9, it indicates no Mycoplasma contamination; if the ratio is between 0.9 and 1.2, the original sample can be cultured for 24-48 h and tested again.

Table 1. Result analysis of Luminescent Mycoplasma Detection Kit

Ratio	Result Analysis	Solution
< 0.9	Negative	No processing required
0.9-1.2	Suspicious	Cells were cultured in isolation for 24-48 h and then tested again
> 1.2	Positive	Dispose of cells after sterilization or isolation using specialized Mycoplasma prevention or removal reagents

Note:

1. The reading time for each well of the multimode reader is set to 1,000 ms, and the reading detection should be strictly carried out after adding Mycoplasma Reagent A for 5 min and Mycoplasma Reagent B for 10 min. Do not advance or delay, otherwise it may affect the analysis of the results of samples with ratios near the critical value.

2. The optimal experimental temperature of this method is 20-25℃, and the reagent must be naturally equilibrated to room temperature before use. Do not heat the reagent in a water bath or other ways. If the reagent needs to be stored for a long time, it is recommended to pack according to the dosage of each experiment and avoid repeated freezing and thawing.

3. The skin surface contains a large amount of ATP, and gloves should be worn when preparing samples and conducting experiments to prevent sample and reagent contamination resulting in false negative or false positive.

4. Some samples, such as the addition of special drugs or the use of some special culture medium, may contain ingredients that inhibit or enhance the reaction and lead to false negative or false positive. At this time, the sample can be diluted 10-fold before testing and further determine whether there is Mycoplasma contamination according to the ratio calculated after dilution.

5. The Ratio of different batches of kits and different instruments used in the same sample will be different, but it will not affect the qualitative judgment.

Typical Data

Table 2. Sample detection data using Luminescent Mycoplasma Detection Kit

Samples	Negative Samples		Positive Samples		Negative Control		Postive Control
	HEK293T cell supernatant	COS-7 cell supernatant	Hela cell supernatant	10-fold dilution	PBS	DMEM	Postive Control
RLU_A	250	241	274	391	302	344	307
RLU_B	80	118	10809	1279	90	109	26484
Ratio	0.32	0.49	39.45	3.27	0.30	0.32	86.27

Other Instructions

Question	Answer
What sensitivity of light detection instrument is recommended for this product?	It is recommended to use a high-sensitivity instrument, if the ratio of multiple detections has been about equal to 1, which may be related to the sensitivity of the detector, the reading time can be adjusted from 1 s to the range of 1-10 s.
How long can prepared Mycoplasma Reagent A solution be stored?	Store at -20℃ away from light, recommended for 3 months, store at -80℃ away from light, effective for 12 months. Separate packaging is recommended to avoid repeated freezing and thawing.
How is luminescence detection different from PCR detection?	Chemiluminescence detection is more rapid, and can detect the presence of mycoplasma contamination in cell culture supernatant at any time and in large quantities.
Is it possible to extend the reaction time and increase the reading for easier calculation?	**No,** strictly follow the operation steps, not in advance or delay, otherwise it will affect the judgment of the ratio near the critical value.
Is it possible to change the sample volume to increase the accuracy of test results?	For 96-well plates, 50 μL samples are recommended, and the sample volume can be increased. At the same time, Mycoplasma Reagent A and Mycoplasma Reagent B are increased proportionally, and the sample size **cannot** exceed 100 μL. It is not recommended to reduce samples, Mycoplasma Reagent A and Mycoplasma Reagent B. The detection value near the background may affect the ratio and result judgment, which may dilute the sample detection.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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