IMMUNOLOGICAL SCIENCES Product Data Sheet

Mycoplasma Detection Kit（Luminescent）

Product name	Cat. No.	Size
Mycoplasma Detection Kit（Luminescent）	IS1901-20T	20T

Descirption/Introduction

This mycoplasma detection kit is designed by using the activity of the specific enzyme in Mycoplasma. This enzyme can decompose the specific substrate in mycoplasma detection reagent and convert ADP into ATP. Luciferase catalyzes the oxidation of luciferin in the presence of ATP to produce biological fluorescence. It can be determined by chemiluminescence instrument to reflect whether the sample to be tested is contaminated by mycoplasma. The whole detection process is simple, only needs two steps, and takes about 15 minutes. This method has high sensitivity and detects Mycoplasma with real biological activity, so the detection result is more accurate than PCR.

Components

Component Number	Component	G1901-20T
ISG1901-1	Mycoplasma Detection reagent A	1 ml
ISG1901-2	Mycoplasma Detection reagent B	1 ml
Manual		1 pc

Assay Protocol / Procedures

1. Take an appropriate amount (1 ml is enough) of cell supernatant cultured for 3-6 days, centrifuge 400 g for 3 min to remove a small amount of floating cells or debris. Take the supernatant and test it immediately, or store it at 4 ℃ for one week, or -80 ℃ for half a year;
2. Balance all test reagents and test samples to room temperature, and the most suitable temperature is 20-25 ℃;
3. Add 50 μL of the sample to be tested, a negative control (e.g., sterile water or PBS) to a 96-well plate (non-transparent plate, a special 96-well white plate is recommended);
4. Add 50μL Mycoplasma Detection reagent A, gently mix it, do not produce bubbles, and keep it away from light at room temperature (20-25 ℃) for 5 minutes. Then the chemiluminescence detection is carried out with an enzyme marker with chemiluminescence detection, and the reading of the meter is A. (Please adjust the corresponding parameters according to the sensitivity of the instrument, and the detection time of each well is generally 0.25-1 s);
5. Add 50μL mycoplasma detection reagent B, gently mix it, do not produce bubbles, and keep it away from light at room temperature (20-25 ℃) for 10 minutes. Then the chemiluminescence detection is carried out with an enzyme marker with chemiluminescence detection, and the reading of the meter is B. (Note: please
test in strict accordance with 10 minutes after adding Mycoplasma test reagent B. It should not be carried out in advance or delayed, otherwise it will affect the judgment of results);
6. Calculate ratio = read value A/ read value B.

A. If B/A>1.1, it indicates that there is mycoplasma contamination in the cell culture;

B. If B/A<0.9, there is no mycoplasma contamination in the cell culture;

7. If the B/A ratio is between 0.9-1.1, it is recommended to continue to culture cells for 24-48 hours, and then test again to determine whether there is mycoplasma contamination. If the b/a ratio is still between 0.9-1.1, the cell culture is not contaminated by Mycoplasma and is Mycoplasma negative.

Note:

1. Mycoplasma detection reagent A contains luciferase, which will be gradually inactivated by repeated freezing and thawing. It is suggested that it should be properly sub packaged and stored after the first thawing, and the sub packaged container should be clean and pollution-free.
2. The use of white or black opaque 96-well plates is strongly recommended for testing, as the use of ordinary transparent 96-well plates may cause interference with neighboring wells.
3. The surface of human skin is rich in ATP. Please wear experimental gloves and masks when testing. Other consumables should also be clean and pollution-free to prevent ATP pollution from external sources.
4. For your safety and health, please wear experimental clothes and disposable gloves.

Storage and Handling Conditions

Ship with wet ice; Stored at -20℃, Mycoplasma Detection reagent A need to keep away from light, valid for 12 months.

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Web-site: www.immunologicalsciences.com - E-Mail: info@immunologicalsciences.com