Mycoplasma LAMP Detection Kit (HNB)

Introduction

Mycoplasma is the smallest prokaryotic microorganism capable of growth and reproduction in non-living culture media, with a size of only 0.1-0.3 μm. It can pass through standard sterilizing-grade filters (0.22-0.45 μm). Mycoplasma contamination alters cellular gene expression and protein function, severely impacting cell physiology. As mycoplasma is not visible to the naked eye, regular testing during cell culture is essential.

There are various methods for detecting mycoplasma contamination, such as culture, PCR, qPCR, and ELISA. However, most are time-consuming and relatively cumbersome to perform. The Mycoplasma LAMP Detection Kit (HNB) utilizes isothermal amplification technology for mycoplasma detection. In the presence of mycoplasma contamination, the conservative sequence of mycoplasma DNA will be specifically amplified by isothermal DNA polymerase, causing the reaction solution to change color from purple-blue to sky blue. The procedure is straightforward and rapid: it requires only 1 μL of culture supernatant and 1-hour incubation at 65°C to generate the result.

Compared to commonly used nested PCR, this kit demonstrates higher tolerance to inhibitors present in the culture supernatant, minimizing the risk of false negatives. The closed-tube format eliminates the need to open the lid after amplification, greatly avoiding cross-contamination and false positives. Results can be determined by direct visual observation, making the kit highly suitable for routine mycoplasma detection in the laboratory.

Components and Storage

Components \ Size	20 Assays	50 Assays	Storage
LAMP Buffer	480 μL	1.2 mL	-20°C
Bst DNA Polymerase	20 μL	50 μL	-20°C
Positive Control	10 μL	25 μL	-20°C
Mineral Oil	500 μL	1.25 mL	-20°C
Shipping: Dry ice	Shelf life: 1 year		

Protocol

1. Sample preparation

    1) For adherent cells, collect the supernatant directly. It is recommended to sample when the cells are

passaged or medium get exchanged for more than 3 days with a cell confluency of about 90%. At this time, mycoplasma concentration in the supernatant is relatively high, facilitating easier detection.

2) For suspension cells, collect the supernatant after centrifugation at 500 g for 5 min. It is recommended to sample when the cells are passaged or medium get exchanged for more than 3 days. At this time, mycoplasma concentration in the supernatant is relatively high, facilitating easier detection.

2. Reaction preparation

1) Warm the LAMP Buffer to room temperature in advance and mix thoroughly by gentle inversion before use. Prepare enough mix for the number of tests according to the table below. Set up a negative control and a positive control in each experiment.

Components	Single reaction volume (μL)
LAMP Buffer	24
Bst DNA Polymerase	1

*Note:

a) Prepare an additional 10 % volume of the mix in order to balance out the losses during pipetting.

b) Laboratory mycoplasma contamination is common. Repeatedly using the LAMP Buffer may lead to contamination from airborne mycoplasma, resulting in false positives. Therefore, it is recommended to handle the reagents in a clean bench with prior UV sterilization of the workspace.

c) To prevent false positives caused by aerosol contamination, it is recommended to use filtered pipette tips and set up the reaction as the following sequence: 1) negative control (seal immediately), 2) test samples, 3) positive control.

2) Mix gently and aliquot 25 μL of mix to each PCR tube or microcentrifuge tube.

3. Adding samples

1) Negative control: add no sample or 1 μL of nuclease free water.

2) Sample: Add 1 μL of cell culture supernatant.

3) Positive control: Add 1 μL of Positive Control.

*Note:

a) For the negative control, adding no sample is preferred. Because self-made nuclease-free water may not be sufficiently pure and can lead to false-positive results.

b) For water bath detection: Add 25 μL of mineral oil to each tube to prevent evaporation. Always change pipette tips between tubes to avoid cross-contamination.

c) For thermal cycler detection: Mineral oil overlay is not required.

4. Reaction

1) For a PCR thermal cycler, run the following program.

25°C	5 min
65°C	60 min
85°C	5 min

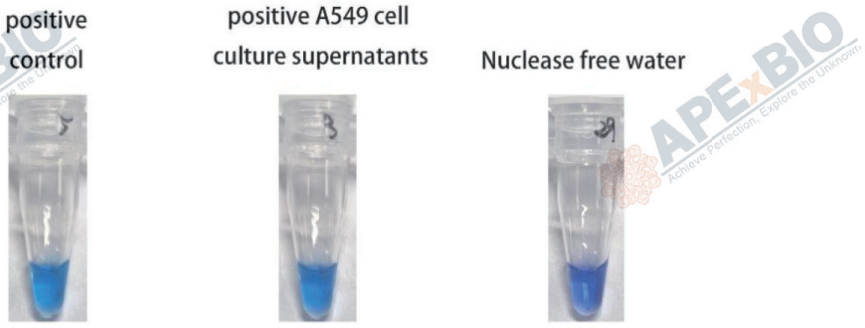
2) For water bath detection, incubate at room temperature for 5 min first. Then, transfer them to a water bath preheated to 65°C and incubate for 60 min.

***Note:**

a) The actual temperature of the water bath may differ from the set temperature, so it is recommended to calibrate it with a thermometer.

b) It is not recommended to use an oven for reactions.

5. Results: Observe the reaction solution color in a bright environment. Using white paper as a background is recommended for optimal visual assessment.



Reaction solution color	**Result**
Purple-blue (keep the original color)	Mycoplasma negative
Sky blue or deep blue (a distinct change compared to negative control)	Mycoplasma positive

***Note:**

a) Do not open the reaction tubes, otherwise the resulting aerosol will cause false positives in subsequent tests. The reaction tubes can be placed in a plastic bag or glove, tied tightly, and disposed of in a special trash can.

b) In rare cases, the negative control may also show a color change due to environmental mycoplasma contamination or the use of home-made nuclease free water. If occurs, perform all procedures inside a UV-sterilized clean bench and use commercially sourced, certified nuclease free water.

Note

1. If mycoplasma contamination is detected, the standard practice is to discard the contaminated culture. For irreplaceable or precious cell lines, mycoplasma eradication reagents (e.g., Cat. No. K2824 or K2825) are recommended. For routine prevention, consider using mycoplasma prevention reagents (e.g., Cat. No. C7202 or C7203).

2. This kit is designed to accurately detect 22 mycoplasma species, including the 8 most common contaminants (*M. laidlawii, M. salivarium, M. hominis, M. pirum, M. arginini, M. orale, M. fermentans, M. hyorhinis*), which collectively account for over 95% of mycoplasma contamination cases in cell culture.
3. The kit incorporates thermolabile UDG to prevent carryover contamination from dUTP-containing amplicons, thereby significantly reducing the risk of false positives and ensuring assay accuracy. The enzyme is active at room temperature and is heat-labile, ensuring its complete inactivation during the subsequent reaction steps without interfering with the results.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.

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