EZassay

Colorimetric Mycoplasma Detection Kit (Phenol Red Method)

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Catalog Code: MYCO-LM-RED-16
MYCO-LM-RED-96

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Brief introduction

The Colorimetric Mycoplasma Detection Kit (LAMP) is designed for the rapid detection of mycoplasma contamination in cell cultures. Based on loop-mediated isothermal amplification (LAMP) and phenol red colorimetric detection, the assay delivers results in approximately 30 minutes without the need for PCR, real-time PCR, electrophoresis, or ELISA. Results are interpreted simply by observing the color change of the reaction mixture.

The kit contains a premixed enzyme and reaction buffer for a convenient one-tube workflow, requiring only the addition of the sample. It is suitable for detecting mycoplasma contamination in culture supernatants from a wide variety of adherent and suspension cell lines, including CHO, HEK293, Vero, Sf9, Hybridoma, and many others.

Targeting the highly conserved 16S rRNA gene, the kit detects the most common mycoplasma species found in cell culture, including but not limited to:

(1) M. hyorhinis, (2) M. fermentans, (3) M. arginini, (4) M. hominis, (5) M. orale, (6) M. salivarium, (7) M. pirum, (8) A. laidlawii, (9) M. pneumoniae, (10) M. bovis, (11) M. bovoculi, (12) A. axanthum, (13) M. buccale, (14) M. agalactiae, (15) M. arthritidis, (16) M. pulmonis, (17) M. gallisepticum, (18) M. gallinarum, (19) M. canis, and (20) Ureaplasma urealyticum.

Materials supplied

Item	MYCO-LM-RED-16	MYCO-LM-RED-96
Mycoplasma Colorimetric Master Mix	400 µL	400µL*6
Positive Control (5X)	10µL	10 µL*6
Mineral Oil	400 µL	400µL*6

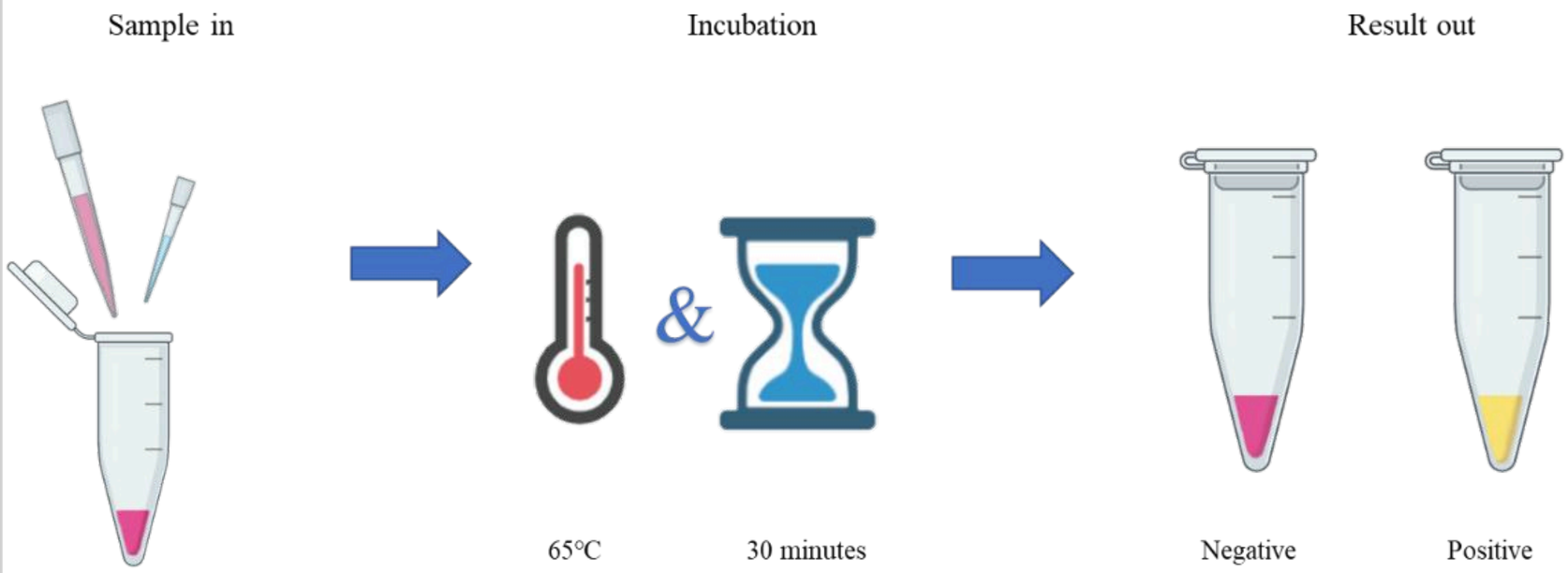
Features

- One-tube colorimetric LAMP assay
- Results in approximately 30 minutes
- Visual readout without specialized instruments
- No PCR, qPCR, electrophoresis, or ELISA required
- Premixed enzyme and reaction buffer for simplified workflow
- Detects a broad range of mycoplasma species targeting the 16S rRNA gene
- Suitable for both adherent and suspension cell cultures
- Ideal for routine mycoplasma screening in cell culture laboratories

Storage

-20°C. Suggest to aliquot after receiving. Avoid repeated freeze-thaw.

Diagram of quick guide



Procedure

1. Prepare sample
- Transfer 200μL cell culture medium to a clean centrifuge tube.
- Heat for 5 minutes at 99 °C. Then cooling down to room temperature.
- Briefly centrifuge and collect supernatant for test.

2. Reaction
- Set up reactions as listed in the following table.

Component	Sample group	Positive control group	Positive control group
Mycoplasma Colorimetric Master Mix	20 μL	20 μL	20 μL
Sample*	5 μL	-	-
Positive control (5X)	-	5 μL	-
ddH2O	Up to 25μL	Up to 25μL	Up to 25μL

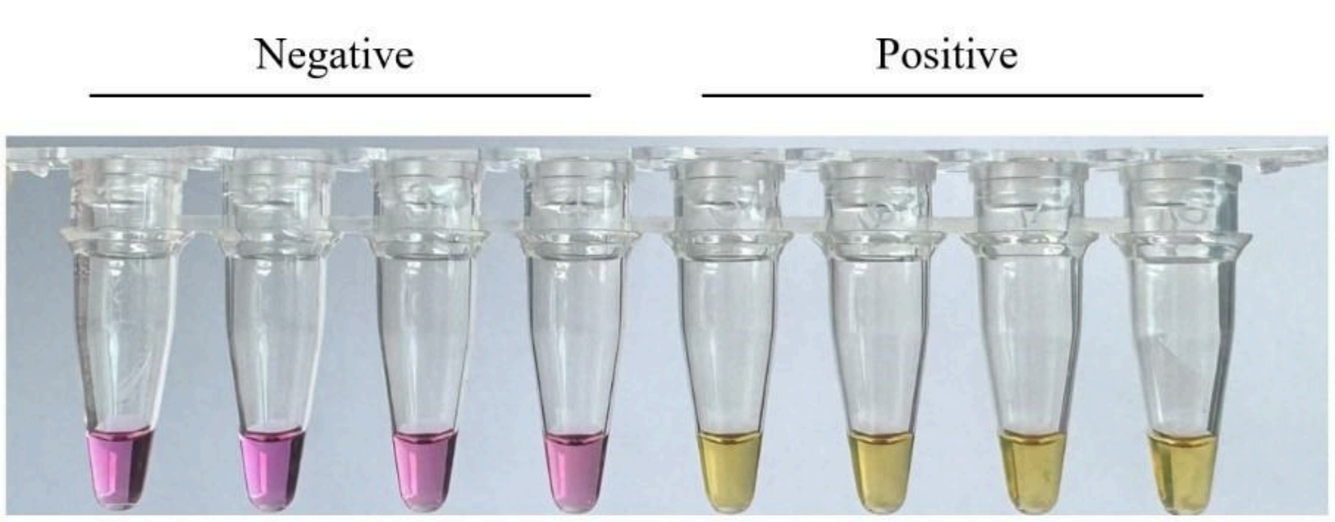
*Sample should be prepared or diluted with distilled water. **Buffer systems such as Tris-HCL should be avoided**. Otherwise, the PH indicator will not change color, which will affect the interpretation of the experimental results.

If using a water bath or heat block, add 20μL of mineral oil to the surface of the thoroughly mixed reaction mixture to prevent evaporation. If using a PCR instrument, set the heated lid temperature to 75 °C; mineral oil is not required.

- Gently flick or up-and-down to mix well. Then quick spin. Incubate the tube at 65 °C for 30 minutes.

3. Result

Cooling down and observe results in a well-lit environment. (recommend white paper as background)



Notes

1. Adherent cells need to be cultured for at least 3 days and have a confluency of 90%. Take the cell culture supernatant for testing.
2. Suspension cells need to be cultured for at least 3 days and centrifuged at 1000 rpm (about 150 g) for 5 minutes. Take the cell culture supernatant for detection.
3. For very low concentrations of mycoplasma, the reaction time can be extended from 30 minutes to 40 minutes. Or follow these steps to enrich for mycoplasma:
① transfer 1mL cell culture medium to a clean tube, centrifuge at 16000 g for 5 minutes，remove the supernatant , remain 50μL, add 200μL PBS or ddH2O.
② 99 °C heat for 5 min，briefly centrifuge, and collect supernatant for testing.
4. This kit employs a pH indicator-based detection method. Because the reaction buffer has a low buffering capacity, the reagent should not be exposed to air for prolonged periods, as it may absorb carbon dioxide (CO_2) from the atmosphere, resulting in acidification of the reaction mixture and a lighter reagent color, which may affect result interpretation. Upon receipt, it is recommended to aliquot the reagent according to the amount required for each use and store at -20 °C. Avoid repeated freeze-thaw cycles.
5. It is recommended to test the positive control and no-template control at least every 1 to 2 weeks.
6. The positive control provided by this kit only contains DNA and will not cause mycoplasma contamination.
7. The sensitivity of the kit is very high, please be careful to avoid carry-over contamination of the amplification product (DNA) to the next test. It is recommended that the sample addition area and result observation area should be operated in separate areas.

Professional supplier of point-of-care test products

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