EZassay

Colorimetric Mycoplasma detection kit (visual detection)

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

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Product Introduction

This kit enables the rapid and simple detection of mycoplasma contamination in cell culture. This novel system detects mycoplasma in half an hour from cell culture supernatant by visual determination eliminating the requirement for PCR, qPCR, electrophoresis or ELISA. It is suitable for mycoplasma detection in a wide range of suspension and adherent cells including CHO, Vero, hybridoma, Sf9, HEK293 and many more.

Targeting the 16S rRNA gene, it covers the most commonly found mycoplasma species in cell culture, including（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、（20）Ureaplasma urealyticum.

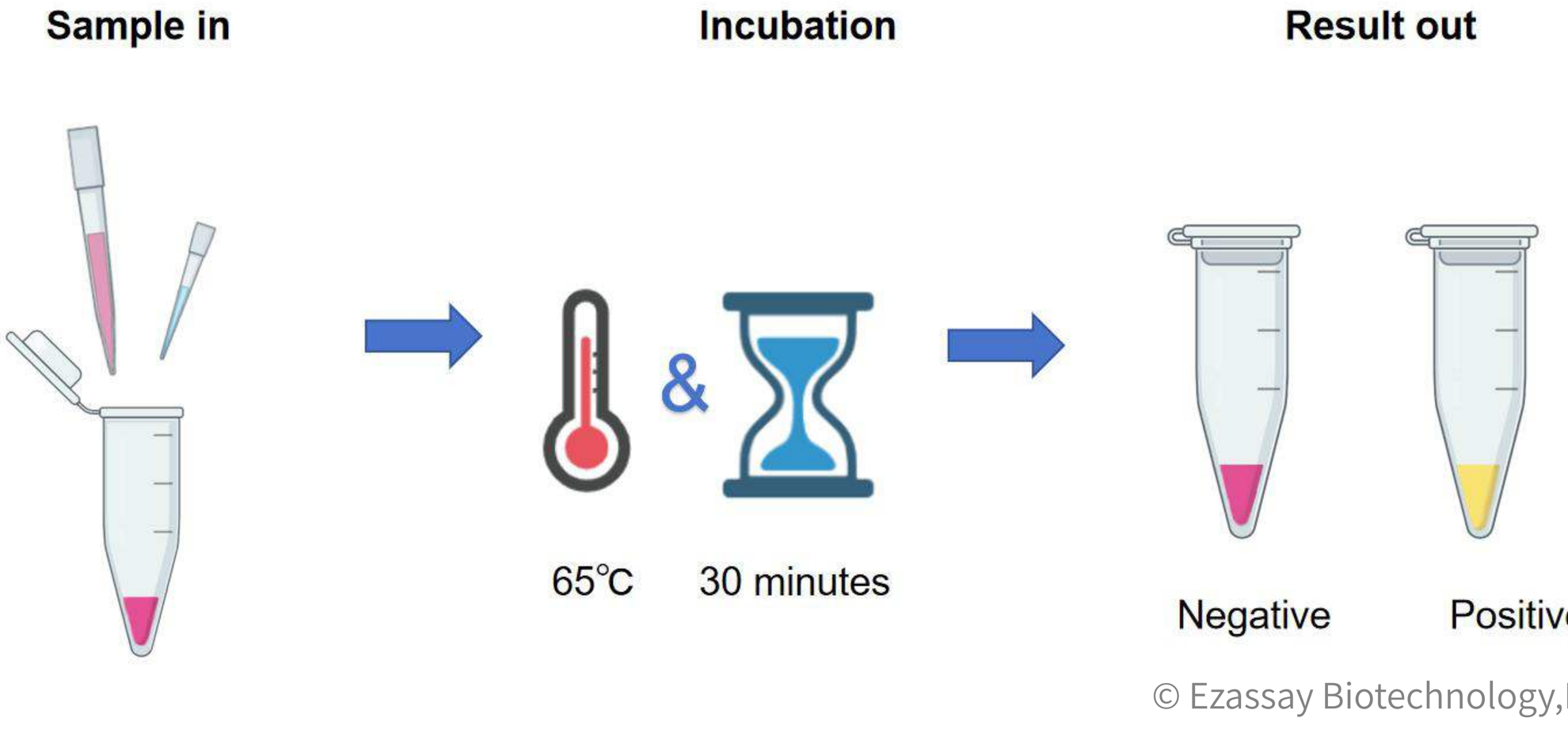
Materials supplied

Component	MYCO-LM-RED-16	MYCO-LM-RED-96
Mycoplasma Dye Mix	400µL	400µL*6
Positive Control DNA (5X)	10 µL	10 µL*6
Mineral Oil	400µL	400µL*6

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.
   - Briefly centrifuge and collect supernatant for test.
2. Reaction

Component	Volume
Mycoplasma Dye Mix	20 μL
Sample or control*	5 μL
Total volume	25 μL

Set up reactions as listed in the following table.

*- 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.
- For negative control, add 5 μL ddH2O.
- For positive control, add 5 μL positive control DNA (5X).

Note. If a water bath is used for incubation, 20 μL mineral oil should be added to each reaction tube to prevent liquid evaporation. If a thermal cycler is used, set the temperature of heated lid to 65 °C, there is no need to add mineral oil.
   - Gently flick or up-and-down to mix well. Then quick spin. Incubate the tube at 65 °C for 30 minutes.

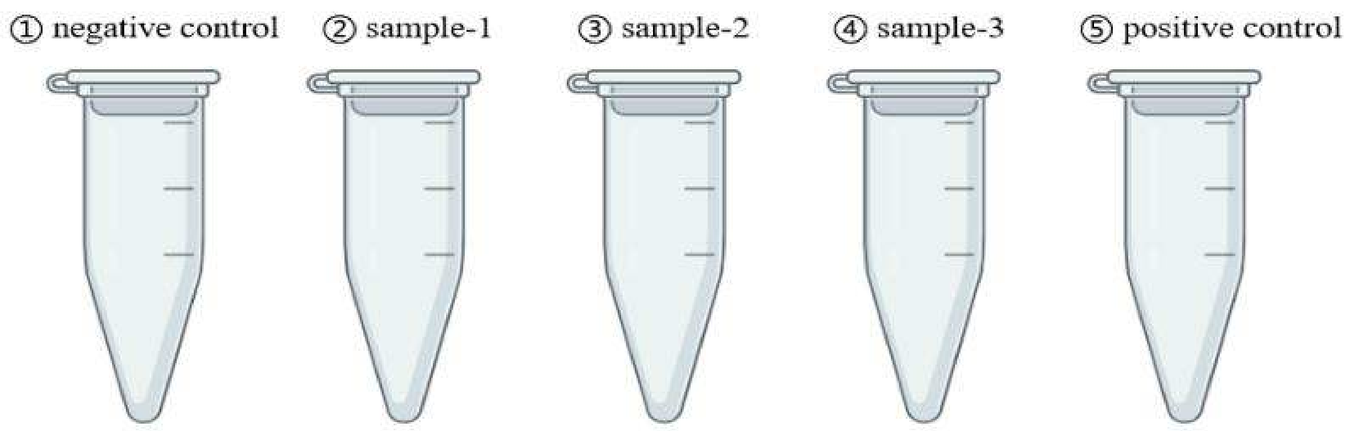


Figure1. To avoid carry-over contamination, it is suggested to perform negative control first, then samples and positive control finally.

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

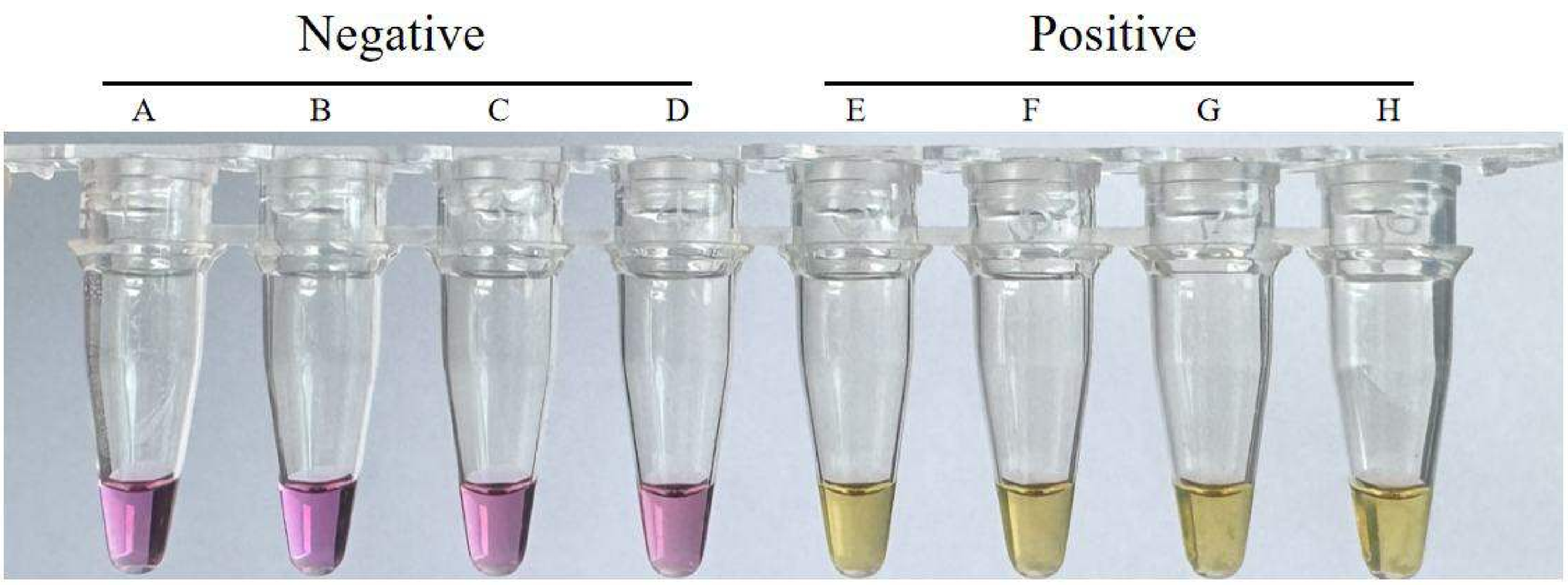


Figure 1. A: ddH2O; B~D: negative samples; E~H: from 5 copies to 5000 copies DNA templates were added per reaction.

Note

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. It is recommended to test the positive control and no-template control at least every 1 to 2 weeks.
5. The positive control provided by this kit only contains DNA and will not cause mycoplasma contamination.
6. 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.