EZassay

Mycoplasma detection paper strip (contamination free)

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EZassay Biotechnology Ltd.

Catalog Code: MYCO- FR-LYO-16
MYCO- FR-LYO-96

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Product Introduction

Combined loop-mediated isothermal amplification (LAMP) with lateral flow paper strip, this kit can detect mycoplasma within 40 minutes. 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. Cross contamination is very likely to happen if you open the lid after amplification. Specially designed tube is used to avoid opening the lid after reaction. It efficiently prevents cross contamination.

Materials supplied

Catalog number	Store	MYCO- FR-LYO-16	MYCO- FR-LYO-96
Reaction Tube ① (with lyophilized powder inside)	-20°C	16T	96T
Reaction Tube ② （with paper strip inside）	Room temperature	16T	96T
Positive Control DNA (25X)	-20°C	10 μL	60 μL
Rehydration Buffer (2X)	-20°C	400 μL	400 μL*6

Materials required but not supplied

1. Dry bath (Compatible with 1.5mL tube)；
2. Pipettes (1~100μL) and clean tips；
3. Distilled water.

Procedure

1. Prepare sample
 • Transfer 200 µL 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

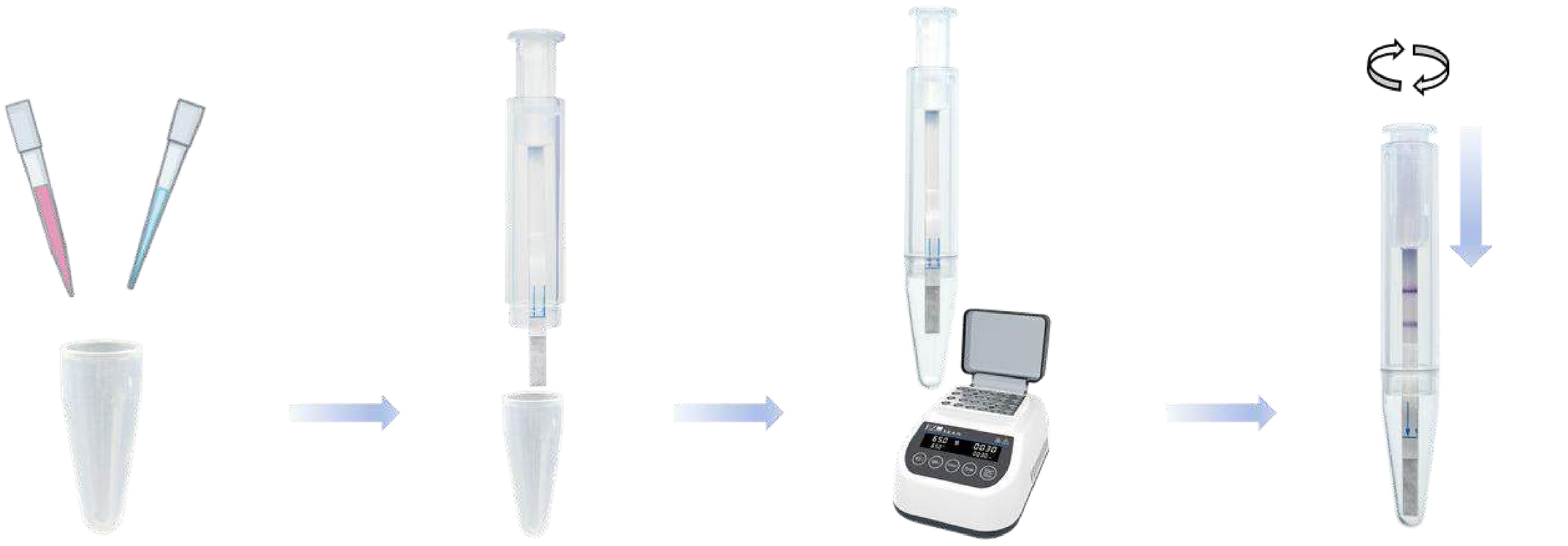
Component	Volume
Rehydration Buffer (2X)	25 µL
Sample*	5~25 µL
dd H2O	Up to 50 µL

• In each reaction tube①, add reagents as listed in the following table.

*- For negative control, add 5 µL ddH2O.
- For positive control, add 5 µL positive control DNA (5X).
• Gently flick or up-and-down to mix well. Then quick spin. Incubate the reaction tube at 65 °C for 30 minutes.

3. Result
• Rotate clockwise the button on the top, press it to allow paper strip to immerse the liquid. Observe the result after 2~5 minutes.

Diagram of quick guide



Comparison of detection results of mycoplasma

Cell culture medium	Culture method (Third-party test results)	Q-PCR method (S company)	Mycoplasma detection paper strip (EZassay Biotech.)	Mycoplasma detection paper strip v.s. Culture method
Sample-1	Positive 	Negative	Positive 	Positive coincidence rate = 100%
Sample-2		Positive 		
Sample-3				
Sample-4				
Sample-5				
Sample-6				
Sample-7	Negative	Negative	Negative	Negative coincidence rate = 100%
Sample-8				

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.