



MycAway™ Plus-Color One-Step Mycoplasma Detection Kit

Product description

The MycAway™ Plus-Color One-Step Mycoplasma Detection Kit is a rapid detection product for mycoplasma contamination in cell culture which developed based on the unique isothermal amplification technology of YEASEN. Compared with the previous mycoplasma detection products, this kit is optimized and has greatly reduced the false positive rate, improved the accuracy of the test and enhanced the identification of negative and positive results. At the end of amplification, the sample can be left at room temperature for a period of time without the change that from negative to positive, and thus will not affect the judgment of the results.

The principle is that if the cell culture is contaminated with mycoplasma, the conserved sequences of mycoplasma DNA will be amplified heavily and rapidly, causing the color of the reaction solution changing from blue-purple to sky-blue, the result is visible to the naked eye, without electrophoresis.

MycAway™ Plus-Color One-Step Mycoplasma Detection Kit can detect multiple mycoplasma species, including eight that are common in cell culture. The traditional nested PCR method for mycoplasma detection is susceptible influenced by the inhibitors in cell culture supernatant and showed a false-negative result. Also if need to perform the electrophoresis, cap opening may increase the contamination and lead to the false-positive result. The one-step mycoplasma detection kit has none of these disadvantages, also its detection sensitivity and accuracy are much higher than those of the PCR method.

Components

Components No.	Name	40612ES25 (25 T)	40612ES60 (100 T)
40612-A	MycAway™ -Color Lamp	425 μL	4 × 425 μL
40612-B	MycAway™ -Color Lamp Primer	50 μL	4 × 50 μL
40612-C	Positive Control	10 μL	4 × 10 μL
40612-D	Mineral oil	500 μL	4 × 500 μL

Shipping and Storage

All the components are shipped with dry ice and can be stored at -15°C ~ -25°C for 18 months. Please keep away from the light.

Instructions

1. DNA extraction

1.1 Adherent Cell: Aspirate the supernatant directly. It is recommended to sample the cell suspension during the cells have been passaged or changed the medium for 3 days or more and the cell fusion level has reached about 90%, When the concentration of mycoplasma in the supernatant is high and easy to detect.

1.2 Suspension Cell: Centrifuge the cells at 500 × g, 5 min, and then aspirate the supernatant. It is recommended to sampling the cell suspension during the cells have been passaged or been changed the medium for 3 days or more and the confluence level has reached to about 90%, When the concentration of mycoplasma in the supernatant is



high and easy to detect.

2. Reaction System

Remove MycAway™ -Color from -20°C, dissolve and then mix upside down, and centrifuge to ensure all liquids are collected to the tube bottom. According to the number of test sample, prepare the following reaction system (usually including the NC and PC).

[Note]: PC is positive control; NC is negative control.

-	Volume (μL)	-	Total Volume (μL)
MycAway™ -Color Lamp	17	× Sample amount	_____
MycAway™ -Color Lamp Primer	2		_____

3. Sample Adding

Test sample: Add 1 μL supernatant of test sample to the reaction vial.

NC: Select the 1st reaction vial which doesn't contain any sample as the NC.

PC: Add 1 μL positive control to the last reaction vial as the PC.

[Note]:3.1 If the reaction is performed in a water bath, add 20 μL mineral oil to each tube to prevent the result errors which due to the liquid evaporation. Mineral oil is not required if the reaction is performed in a PCR instrument.

3.2 As this is a sensitive kit and Mycoplasma contamination is commonly in the general laboratory environment, so we advice users to prepare the reaction systems in the clean bench and reduce the cap opening times to avoid false-positive results.

4. Reaction Condition

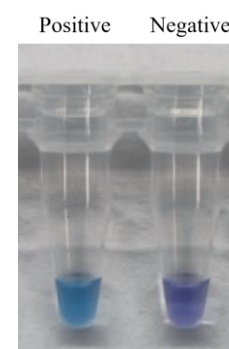
After the samples are added, incubate at 30 °C for 5 min, then heat at 63°C for 60 min.

[Note]: The enzyme is very sensitive to the temperature, so it is highly recommended to operate with a PCR instrument; If a water bath is used, it should be preheated to the specified temperature. If the temperature deviated from the target by 2°C or more, then the amplification efficiency will be reduced and the positive reaction may not reach to the typical sky blue color in specified time.

5. Results Judgement

After the final incubation, take out the vials, and place at room temperature. Observe the reaction results in a well-lit environment (white paper is recommended as a background). If the reaction solution remains blue-purple, it is negative; if the reaction solution is sky blue, it is positive.

[Note]: The incubation time at 63°C should be strictly controlled or a false positive result will appear. Do not open the cap of the reaction tube, or the mycoplasma in the environment will lead to a false positive result.



Figures 1. Detection Result

Notes

1. Please read this manual carefully before using this kit.
2. The whole experiment should be operated in on the clean bench with a standardized manner, includes the preparation of reaction system, sample processing and sample addition.
3. For your safety and health, please wear lab coats and disposable gloves for operation.
4. This product is for research use ONLY!