Mycoplasma Detector

CAT NO.: APL-5002-1 / APL-5002-2

DESCRIPTION

Mycoplasma Detector is a ready-to-use reagent for rapid detection of Mycoplasma contamination in cell cultures. Add 1 µL of the cell culture supernatant into the reaction system and incubate at 60 °C for 1 hour, then the results could be observed by naked eyes. *Mycoplasma detector* can be done easily in the cell culture room without PCR/qPCR and electrophoresis process. Compared with the PCR method, Mycoplasma Detector is more resistant to the culture medium inhibitors, so there is no false positives and false negatives results. The detection results are highly consistent with the most sensitive and accurate qPCR methods. Mycoplasma Detector can detect 28 kinds of mycoplasma, including 8 kinds of mycoplasma commonly found in the cell culture process. Mycoplasma Detector is suitable for suspension and adherent cells, including CHO, VERO, Hybridoma, SF9, HEK293 etc., with a wide range medium compatibility.

CONTENT

Components	APL-5002-1 (20 rxns)	APL-5002-2 (50 rxns)
Mycoplasma detector buffer	480 µL	1.2 mL
Mycoplasma detector enzyme	20 µL	50 µL
Positive control	10 µL	25 µL
Paraffin oil	500 µL	1.25 mL

STORAGE

This product should be stored at -20°C and avoid repeated freeze-thaw cycles to retain the maximum activity.

OTHER MATERIALS

Water bath or PCR machine (for hot plate)

PROTOCOL

1. **Obtain cell culture supernatant:**

   1.1. Adherent cells: Aspirate the supernatant directly. We recommend the cell subculture or medium exchange through 3 days later for the detection and the cell density is 90% confluence.

   1.2. Suspension cells: Centrifuge with 2,300 rpm 5 min then aspirate the supernatant. We recommend the cell subculture or medium exchange through 3 days later for the detection.

2. **Reaction system preparation:**

   2.1. Thaw the Mycoplasma detector buffer and mix the buffer via inverting several times. According to the sample numbers, preparing the reaction system in a microcentrifuge tube below.

Components	Volume with one reaction (μL)	
Mycoplasma detector buffer	24	x Sample number a x 1.1b
Mycoplasma detector enzyme	1	

a. One negative and positive control to each experiment is recommended.

b. Prepare the 1.1 times reaction mixture volume for the pipetting loss.

2.2. Gently pipetting and dispense into the microcentrifuge tubes, each tube is 25 μL reaction mixture.

3. Sample preparation

Sample: Add 1 μL culture medium into the reaction tube

Positive control: Add 1 μL positive control

Negative control: Do not add any solution or add 1 μL sterile water

* If the reaction is in a water bath, add 25 μL paraffin oil to each tube to prevent liquid evaporation. When adding paraffin oil, please change the tip to prevent cross-contamination between samples.

* If the reaction is in a PCR machine, paraffin oil is not required.

4. Reaction

Set 60°C of the water bath or PCR machine then incubate the reaction tube for 60 min.

* The actual temperature of the water bath may deviate from the set temperature. It is recommended to calibrate with a thermometer first. The reaction can be carried out at 58 ~ 64°C, but it will affect the detection sensitivity.

* It is not recommended to use an oven for the reaction.

5. Results measurement

If the color of the reaction mixture is purple, it is mycoplasma-negative. If the color changes to sky blue, it is mycoplasma-positive. In a few cases (such as low mycoplasma content), the color is between purple and sky blue, in which case the reaction time can be extended to 75 - 90 min. If the color changes to sky blue, it is mycoplasma-positive, otherwise it is mycoplasma-negative. The color is shown in the below:

Mycoplasma-negative

Mycoplasma-positive

* DO NOT open the lid during the reaction, otherwise the generated aerosol may result false-positives in subsequent tests.

* It is recommended to wrap the reaction tube in a plastic bag or gloves, discard it in a special trash can, and clean it up in time.

PRODUCT USE LIMITATION

Research use only.