MycoGenie Rapid Mycoplasma Detection Kit

Code: MP90020
Pack Size: 20 tests

Code: MP90050
Pack Size: 50 tests

1. Introduction

MycoGenie Rapid Mycoplasma Detection Kit is designed for rapid detection of mycoplasma contamination in cell culture. It's easy to use: after adding 1 µl of the cell culture supernatant to the reaction system and incubated at 60°C for 1 hr, the results can be determined by visual observation. Detection can be easily completed in cell room without doing PCR, qPCR, or electrophoresis. Compared with conventional PCR methods, MycoGenie Mycoplasma Detector is more resistant to the inhibitor in the culture supernatant, which avoids weak positive and false negative. There's no need to do electrophoresis after reaction, which avoids false positive from aerosol of amplification product. The result is highly consistent with the most sensitive and accurate PCR method.

It has been validated that MycoGenie Rapid Mycoplasma Detection Kit could detect up to 28 kinds of mycoplasma, including 8 kinds commonly found in cell culture. This kit is suitable for mycoplasma detection in many kinds of suspension and adherent cells, including CHO, Vero, hybridoma, Sf9, HEK293, etc. This kit has a wide range of media and serum compatibility. It's suitable for routine mycoplasma detections in biopharmaceutical companies, vaccine/monoclonal antibody manufacturers, cell therapy/embryo laboratories, and other scientific research laboratories.

2. Kit Components

Components	MP90020 – 20 tests	MP90050 – 50 tests
MycoGenie Buffer*	480 µl	1.2 ml
MycoGenie Enzyme	20 µl	50 µl
Positive Control	10 µl	25 µl
Paraffin Oil	500 µl	1.25 ml

*Contains chromogenic reagent.

3. Storage

Store at -30 ~ -15°C and transport at ≤0°C

4. Materials Needed But Not Supplied

PCR instrument or water bath.

5. Application

MycoGenie Rapid Mycoplasma Detection Kit is suitable for many kinds of suspension and adherent cells with a wide range of media and serum compatibility, which include but do not limit to:
Suspension cells: CHO, NS0, 293F, mouse hybridomas, Sf9, BHK21, etc.
Adherent cells: Vero, MDCK, SP2/0, 293T, HepG2. HeLa, A549, MB-MDA231, L929, MEF, etc.
Medium: CD FortiCHO, CDM4, Expi 293 Medium, CD Hybridoma, Grace, DMEM, 1640, F12, etc.
Serum: Fetal bovine/calf serum, horse serum, Gibco KSR serum replacement, etc.

6. Protocol

1. Collect the cell culture supernatant

For adherent cells: directly collect the supernatant. **It is recommended to collect the sample when the cells are passed or medium is exchanged for more than 3 days with a cell confluence of about 90%**. At this moment, mycoplasma content in supernatant is relatively high and easy to be detected.

For suspension cells: collect the supernatant after centrifugation at 2,300 rpm (500 × g) for 5 min**. It is recommended to collect the sample when the cells are passed or medium is exchanged for more than 3 days.** At this moment, mycoplasma content in supernatant is relatively high and easy to be detected.

2. Preparation of reaction system

Thaw the MycoGenie Buffer and mix thoroughly. Prepare following reaction system in a microcentrifuge tube:

Components	Volume for a Single Reaction	
MycoGenie Buffer	24 µl	x Number of Samples[a] x 1.1[b]
MycoGenie Enzyme	1 µl	

Note: a. Set a negative control and a positive control for each experiment, if necessary.
b. The extra 10% volume of solution is necessary to ensure that sufficient quantities can be divided into each tube, due to pipetting errors.

Gently mix by pipetting then aliquot 25 µl of solution to each PCR tube or microcentrifuge tube.

3. Adding Samples

Sample: add 1 µl of supernatant to be detected to each reaction tube.
Positive control: add 1 µl of Positive Control.
Negative control: add 1 µl of sterile water as a negative control

Note: If the reaction is carried out in a water bath, add 25 µl of Paraffin Oil to each tube to prevent liquid evaporation. When adding Paraffin Oil, please change tips between samples to prevent cross-contamination.
Note: If the reaction is carried out in a PCR instrument with a hot lid, it is not necessary to add paraffin oil.

4. Reaction

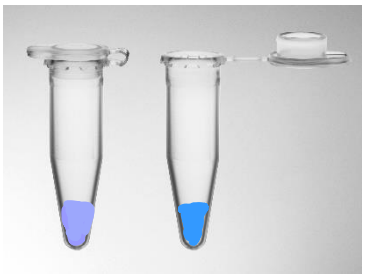
Incubate at 60°C for 60 min in a PCR instrument or water bath.
Note: The actual temperature of the water bath may deviate from the set temperature. It is recommended to calibrate it with a thermometer first. In fact, 58-64°C is also acceptable, but it will affect the detection sensitivity.
Note: It is not recommended to use an oven for this reaction

5. Results

Observe the solution color in a bright environment. It is recommended to use a white paper as background. Purple solution color represents mycoplasma negative and sky blue color represents mycoplasma positive (as shown in figure on the right).

In a few cases (i.e. low mycoplasma content), the color may be between purple and sky blue. Under this circumstance, extend the reaction time to 75 min-90 min and observe the color again. The negative control or positive control can also be used as references.

Note: The reaction tube should not be opened, due to possible false positives in the subsequent detection resulted from aerosol. Wrap the reaction tube in a plastic bag and discard it into trash in another room and clean up on time.



Negative Positive

FAQ & Trouble Shooting

1. What is the sensitivity of MycoGenie Mycoplasma Detector? How to ensure the detection sensitivity?

The detection sensitivity is 5×10^5cfu/ml. Generally, the mycoplasma content in culture supernatant is 10^6– 10^8cfu/ml. As previously reported, one single mycoplasma in cell culture can grow to 10^6cfu/ml in 3 - 5 days. Therefore, cells should remain in culture for at least 72 h undisturbed prior to screening.

2. The color changes as soon as adding the supernatant. Or the solution turns to other colors other than purple and blue after reaction.

In rare cases, ingredients of the medium interfere with the color of the MycoGenie reagent. For example, the Cell Boost 5 (Hyclone) makes the MycoGenie reagent appears pink. To avoid this,

(1) Collect a small amount of culture supernatant or cell suspension and centrifuge at 2,300 rpm (500 × g) for 5 min. Collect the supernatant.

(2) Centrifuge again with a high speed (>11,200 rpm (12,000 × g)) for 5 min to precipitate mycoplasma in the supernatant. Discard most of the supernatant and leave about 50 µl in the tube. Add 950 µl of sterile water and mix gently by pipetting.

(3) Repeat the Step (2) for three times. Discard most of the supernatant and leave about 50 µl in the tube.

(4) Take 1 µl of supernatant for detection.

3. How to save cells from mycoplasma contamination?

If mycoplasma contamination occurs, it is recommended to discard the cells to prevent other cells from contamination. If mycoplasma positive is detected, the same batch of cells should be discarded.

4. How to avoid false positives?

Generally, no false positives will occur during proper operations. DO NOT open the tubes after reaction, due to possible false positives in the subsequent detection resulted from aerosol. Change tips between samples, and the positive control should be added at last. These operations can further reduce the risk of false positives.

5. How many kinds of mycoplasma can be detected by MycoGenie Mycoplasma Detector?

There are 28 kinds of mycoplasma that can be detected accurately by MycoGenie Mycoplasma Detector:

A. laidlawii*	M. salivarium*	M. neophronis	M. primatum	M. gallinarum	M. lipophilum
M. hominis*	M. pirum*	M. timone	M. leopharyngis	M. sphenisci	M. falconis
M. arginini*	M. orale*	M. caviae	M. maculosum	M. bovigenitalium	M. alkalescens
M. fermentans*	A. granularum	M. alvi	A. oculi	M. auris	
M. hyorhinis*	A. pleciae	M. bovis	M. iners	M. columbinum	

* More than 95% mycoplasma contaminations in cell culture are caused by these 8 kinds of mycoplasma

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