MycoBlue Mycoplasma Detector

D101

Version 22.1

Product Description

MycoBlue Mycoplasma Detector is designed for rapid detection of mycoplasma contamination in cell culture. Its advantage is convenience, after adding 1 µl of the cell culture supernatant to the reaction system and incubating at 60℃ for 1 h, the results can be determined by visual observation. Detection can be easily completed in cell culture lab without performing PCR, qPCR, or electrophoresis. Compared with conventional PCR methods, MycoBlue Mycoplasma Detector is more resistant to the inhibitor in the culture supernatant, avoiding weak positive and false negative. There is no need to perform electrophoresis, avoiding false positive from aerosol contamination. The result is highly consistent with the most sensitive and accurate qPCR method.

It has been validated that MycoBlue Mycoplasma Detector could detect up to 28 kinds of mycoplasma, including 8 commonly found strains in cell culture. This product is suitable for mycoplasma detection in various types of suspension and adherent cells, including CHO, Vero, hybridoma, Sf9, HEK293, etc. It has a wide range of cell culture medium compatibility. MycoBlue Mycoplasma Detector is applicable for routine mycoplasma detection in biopharmaceutical companies, vaccine/monoclonal antibody manufacturers, cell therapy/embryo laboratories, and other scientific research laboratories.

Components

Components	D101-01 20 rxns	D101-02 50 rxns
MycoBlue Buffer*	480 µl	1.2 ml
MycoBlue Enzyme	20 µl	50 µl
Positive Control	10 µl	25 µl
Paraffin Oil	500 µl	1.25 ml

* It contains chromogenic reagent.

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Self-prepared Materials

PCR instrument or water bath.

Notes

For research use only. Not for use in diagnostic procedures.

Tighten the cap of the tube during storage to keep it sealed.

Applications

It is applicable for various types of suspension and adherent cells with a wide range of cell culture medium 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.

Cell culture 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.

Experiment Process

1. Collect the cell culture supernatant

For adherent cells: directly collect the supernatant. **Cells should remain in culture for at least 72 h undisturbed prior to screening and reach 90% confluence.** At this moment, mycoplasma content in supernatant is relatively high and can be detected easily.

For suspension cells: collect the supernatant after centrifugation at 2,300 rpm (500 × g) for 5 min. **Cells should remain in culture for at least 72 h undisturbed prior to screening.** At this moment, mycoplasma content in supernatant is relatively high and easy to be detected.

2. Preparation of reaction system

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

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

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 transfer 25 µl of reaction mix 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 Nuclease-free ddH_2O as a negative control.

▲ If the reaction is carried out in a water bath, add 25 μl of Paraffin Oil to each tube to prevent inaccurate results due to liquid evaporation. Please pay attention to replace the tip when adding Paraffin Oil to prevent cross-contamination between samples.

▲ If the reaction is carried out in a PCR instrument, Paraffin Oil is not required.

4. Reaction

Incubate at 60℃ for 60 min in a PCR instrument or water bath.

▲ 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℃ is also acceptable, but it will affect the detection sensitivity.

▲ 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 represents negative and sky blue represents positive (as shown in the figure below). In some circumstances (such as low mycoplasma content), the color may be between purple and sky blue. Then extend the reaction time to 75 - 90 min and observe the color again. The negative control or positive control can also be used as references.

Negative Positive

▲ Do not open the lid of detection tubes to prevent false positives due to aerosol contamination. It is recommended to wrap the reaction tube in a plastic bag and discard it to a dedicated trash can.

FAQ & Troubleshooting

◇ What is the sensitivity of MycoBlue Mycoplasma Detector? How to ensure the detection sensitivity?

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

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

In rare cases, ingredients of the medium interfere with the color of the MycoBlue reagent. For example, the Cell Boost 5 (Hyclone) makes the MycoBlue reagent turn 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.

◇ How to avoid false positives?

Do not open the lid of detection tubes to prevent false positives due to aerosol contamination. Change the tips and add Positive Control at last.

◇ 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 the cells are precious, try to use Myco-Off Mycoplasma Cleaner (Vazyme #D103) to save the cells.

◇ How many kinds of mycoplasma can be detected by MycoBlue Mycoplasma Detector?

There are 28 kinds of mycoplasma that can be detected accurately by MycoBlue 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.