MycoBlue™ Mycoplasma Detector

Catalog # D101

Version 5.1

Vazyme biotech co., ltd.

Introduction

MycoBlue™ Mycoplasma Detector is a rapid detection of mycoplasma kit for cell culture. Its core technology is unique isothermal amplification of Vazyme. MycoBlue™ Mycoplasma Detector is simple and convenient. Only 1 μl cell culture supernatant need to be added to the reaction system, and incubated at 60 C for 1 hour. If the cell culture is contaminated by Mycoplasma, The conserved sequence of Mycoplasma DNA was amplified by isothermal DNA polymerase, which made the reaction liquid from purple to blue. The results can be determined by the direct observation. No need for the qPCR or PCR instrument and electrophoresis, all the experiments can be easily completed in the cell culture room. MycoBlue™ Mycoplasma Detector can detect as much as 28 kinds of mycoplasma, including 8 kinds of Mycoplasma which is common in cell culture. Compared with commonly regular PCR, MycoBlue™ Mycoplasma Detector is more tolerance to the inhibitor in the culture supernatant and there is no weakly positive and false negative. There is no need to open after the reaction which avoids the false positive results caused by the expansion of the aerosol. The results of detection are in high agreement with the most sensitive and accurate quantitative PCR method. MycoBlue™ Mycoplasma Detector is suitable for all kinds of suspension and adherent culture cells, including CHO, Vero, hybridoma, Sf9, and 293, etc. MycoBlue™ Mycoplasma Detector is also compatibility with widely used culture medium and serum. As a result, MycoBlue™ Mycoplasma Detector is very suitable for the daily mycoplasma detection of Bio pharmaceutical companies, vaccine manufacturers, monoclonal antibodies production enterprises, cell therapy / embryo laboratories and scientific research.

Package Information

Component	D101-01 20 tests	D101-02 50 tests
MycoBlue™ A[a]	480 μl	1.2 ml
MycoBlue™ B[b]	20 μl	50 μl
Positive Control	10 μl	25 μl
Paraffin oil	500 μl	1.25 ml

a.Including buffer and chromogenic agent.

b.Including isothermal polymerase.

Storage

Store at -20℃.

Protocol

1.Collect cell culture supernatant.

Adherent cell Directly absorb the supernatant. It is suggested that cell lines should be precultured for 3 days for several days to maximize test sensitivity.

Suspension cell centrifugate (500 g) for 5 min and absorb the supernatant. It is suggested that cell lines should be precultured for 3 days for several days to maximize test sensitivity.

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2.Prepare the reaction system.

Take out the MycoBlueTM from -20℃, melt and mix gently. Prepare the following reaction system in a micro centrifuge tube dependent on the number of the tested sample (As need, add on negative control and positive control every experiment).

	Single reaction volume (μl)		Total volume (μl)
MycoBlue™ A	24	×Number of sample ×1.1	-
MycoBlue™ B	1		-

There are pipetting errors and the 10% is to ensure that there is enough amounts to be assigned to the packaging per tube.

Mix gently with a pipetman. Put into PCR tubes or microcentrifuge tubes and each tube is 25 ul.

3.Add sample.

The first reaction tube does not contain any sample as a negative control;

Add 1 μl to the rest of the tested culture supernatant reaction tube;

Add 1 μl Positive Control to the last tube as positive control.

If the reaction is carried out in water bath, add 25 ul paraffin oil to each tube in order to prevent evaporation of liquid which will lead to an inaccurate result. Please change the tips to prevent cross contamination between the samples. Do not add any paraffin oil if the reaction is carried out in the PCR meter.

4.Reaction.

Incubate at 60℃ for one hour in water bath kettle or PCR meter.

The actual temperature of bath may be deviate from the set temperature. A simple calibration with mercury thermometer is recommended. In fact, 58-64℃ reaction can be carried out, but the sensitivity of the detection will be no more than 60℃.

5.Result determination.

Observe the color of reaction system in a in a well light environment (white paper as background is recommended). If the color of the solution is still purple, the result is judged to be Mycoplasma negative; if the solution turns blue room purple, then the result is sentenced to be Mycoplasma positive. In some cases (e.g., lower level of Mycoplasma infection), the color of the solution may be between purple and blue and the reaction time need to be extended to 75-90 minutes. If the solution becomes to be blue, it is judged to be positive otherwise it is negative. The color is shown in the following figure which also can be referenced to the negative control and positive control.

Notes: The reaction product should not be opened! Otherwise the false positive results will be caused by the expansion of the aerosol. The reaction tube should be wrapped in a plastic bag or a pair of gloves and dropped into a garbage barrel in another room, and cleaned up in time.

Negative

Positive

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6.Remedial measures for Mycoplasma Contamination.

If there are mycoplasma contaminations in the cell culture medium, it is recommended to discard the cells to prevent other cells from being polluted. At the same time, recover a same batch of frozen cells, and take a detection of mycoplasma. If the result is positive, discard the whole lot of the cell. If the cells are very precious, try to use Myco-Off™ (Vazyme D102-01), to save the cells.

Trouble Shooting

Q. What is the sensitivity of MycoBlue™ Mycoplasma Detector? How to guarantee the sensitivity of detection?

A: In general, the Mycoplasma in the culture supernatant was between 106 and 108 cfu/ml. MycoBlue™ Mycoplasma Detector can detect 500 CFU of Mycoplasma in 1μl supernatant which means that the sensitivity is less than 5×105 cfu/ml. MycoBlue™ Mycoplasma Detector can meet the needs of the most experiments. According to the article, a single mycoplasma infection can amplify to 106 cfu/ml in 3-5 days. Therefore, in order to improve the detection rate, try to determine the culture medium which has been exchanged for three days.

Q. After the addition of the culture supernatant, the reaction liquid changes the color immediately; or the reaction liquid color emerges beyond purple and blue. What is the reason? How to solve the problem?

A: In few cases, the components of the medium may interfere with the color of MycoBlue™ reagent. For example, we found that Boost Cell 5 (Hyclone) will make the MycoBlue™ reagent exhibit Pink. Under such circumstance, centrifuge (500 g) the culture medium for 5 min and collect the supernatant. Nest centrifugate (>12000 g) for 5 min and Precipitate the possible presence of Mycoplasma in the supernatant. Remove most of the supernatant and keep about 50 ul supernatant. Then add 950 ul of sterile water, mix with pipette, nest take a high speed centrifugation (>12000 g) for 5 minutes, remove most of the supernatant and leave about 50 ul water. Then add another 950 UL of sterile water, mix with pipette, nest take a high speed centrifugation (>12000 g) for 5 minutes, remove most of the supernatant and leave about 50 ul water. Finally, take 1 ul water to detect.

Q. How to judge the color accurately? How to take the pictures for the results and save?

A: Please make sure that the color of the reaction liquid is observed in a light environment. Negative, positive color is the best reference. If there is no control, you can also compare the results with the previous photos. It is recommended to take the same angle, brightness, and white as the background for the pictures every time.

Q. Is there any false positive?

A: There will not be false positive as long as you pay attention to the operations. The most important things is that do not open the lid, otherwise the reaction products will produce aerosol, which leads to false positive results. In addition, change the tips every time and add the positive control in the last tube can further reduce the risk of false positive.

Q. Which cell/ culture medium are suitable for the Mycoplasma detection with Mycoplasma Detector

A: MycoBlue™ Mycoplasma Detector is suitable for all kinds of suspension and adherent cells, and also has an extensive compatibility with the culture medium and serum. We have verified the cells, culture medium and serum including (but not limited):

Suspension adherent	CHO, NS0, 293F, Mouse hybrid tumor cells, Sf9, BHK21 Etc.
Adherent cells	Vero, MDCK, SP2/0, 293T, HepG2, Hela, A549, MB-MDA231, L929, MEF etc.
Culture medium	CD FortiCHO, CDM4, Expi 293 Medium, CD Hybridoma, Grace, DMEM, 1640, F12 etc.
Serum	Fetal / new born serum; horse serum; Gibco KSR serum replacement etc.

Q. How many kinds of mycoplasma can be detected by MycoBlue™ Mycoplasma Detector?
A: According to statistics, more than 95% of the cells are contaminated by 8 kinds of mycoplasmas. MycoBlue™ Mycoplasma Detector can detect as many as 28 kinds of mycoplasmas, including 8 kinds of Mycoplasmas which is common in cell culture.

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	

Mycoplasma Contamination, Prevention and Elimination

Source of Mycoplasma Contamination:

Work environment, researcher itself (some of mycoplasma are normal microbial flora for human, such as mycoplasma orale), culture medium (in particular serum), cross contamination by contaminated cell, experiment equipment, original tissue or organ for cell preparation, etc. cell cultures contaminated by mycoplasmas not always turn to turbid, and easy to be ignored. Mycoplasma contamination can lead to various problems, including the following aspects: changes in cell growth rate; induced changes in cell morphology; chromosome aberration; alter cell metabolism; decreased the survival rate after cell recovery; reduce the transfection efficiency; abnormal amount of gene expression.

Mycoplasma Prevention:

1. Strictly control the cell culture environment, incubator and shaker should be regularly disinfected (with alcohol or UV); to ensure water (water bath, water dish of Incubator) is clean;
2. Ensure aseptic operation, reduce the talk, and wear a mask (to prevent mycoplasma orale contamination);
3. Purchase high quality and reliable serum;
4. Foreign cells must first be detected without mycoplasma contamination before they can enter the laboratory;
5. It is very important to do mycoplasma detection of cell culture regularly. In general, mycoplasma detection should be taken every 1-2 weeks. Routinization and persistence of regularly detection is the key to deal with mycoplasma infection for cell culture laboratory.

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Mycoplasma Elimination:

Most of the antibiotics used in cell culture are ineffective for Mycoplasma. It is recommended that the mycoplasma contamination cells should be discarded directly to prevent the contamination of other cells. If the cell is very precious, try to use mycoplasma removal reagent Myco-Off™ (Vazyme D102-01), to rescue the cells.

References:

1. Drexler et al., Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology 2002: 39, 75-90.

2. McGarrity et al., Mycoplasmas, molecular biology and pathogenesis. Washington DC: American Society for Microbiology 1992:445-454