Myco-Clear Mycoplasma Removal Agent

Introduction

Mycoplasmas are the smallest prokaryotic microorganism, only 0.1-0.3 μm in size. Due to their small size, mycoplasmas can penetrate rated filters (0.22~0.45 μm). Mycoplasma contamination remains a major problem in cell culture. Mycoplasmas can alter the DNA, RNA, and protein synthesis of culture cells, but they may not noticeably affect cell growth rates in many cases. And it is a must to eliminate mycoplasma contamination for cell culture.

The commonly used mycoplasma removal reagents are mainly tetracycline, macrolide, and quinolone antibiotics. These antibiotics can only inhibit mycoplasma, but they do not kill mycoplasma directly, so they are slow to act and not long-lasting. Resistance strains can also be developed after prolonged use.

The main active ingredient of Myco-Clear Mycoplasma Removal Agent is a surfactant peptide extracted from fermented products of Bacillus subtilis, which can selectively bind to mycoplasma membranes and change the permeability of mycoplasma membranes, thereby directly killing mycoplasma. At the same time, it cannot cross the cell membrane of eukaryotic cells, so it does not affect the characteristics of the cell. During use, this reagent may affect the proliferative ability of cells, but the cells can return to their normal proliferative state after removing this reagent.

This reagent is a ready-to-use, sterile solution, and can eliminate mycoplasma in cells in 5-8 days. Compared with traditional antibiotic products, this reagent can directly kill mycoplasma, with better and more complete elimination, and the possibility of mycoplasma resistance is lower. For faster and simpler elimination of mycoplasma, we recommend using Myco-Clear Mycoplasma Removal Agent Plus (Cat. No. K2825), an upgraded version of this reagent.

Components and Storage

Components \ Size	4 mL	20 mL	Storage
Myco-Clear Mycoplasma Removal Agent	4 mL	20 mL	4°C
Shipping: Blue ice	Shelf life: 1 year		

Protocol

1. **Adherent cell treatment**

1) Trypsinize cells in fresh medium containing 5% fetal bovine serum with a cell density of 5,000-50,000 cells/mL.

***Note:**

a) Before using this reagent, make sure that the cells are all trypsinized into single cells and do not form clumps. This is because this reagent requires direct contact with mycoplasma to work. Clumping of cells can be avoided by extending the trypsinization time.

b) High concentrations of fetal bovine serum can affect mycoplasma elimination. In the process of mycoplasma removal, it is recommended to use fetal bovine serum at a concentration of 5%.

2) Add 2.8 mL of fresh medium containing 5% fetal bovine serum to a 6 cm plate, add 200 µL of Myco-Clear Mycoplasma Removal Agent, and mix.

3) Add 2 mL of the cell suspension prepared in step 1 and mix. At this point, the number of cells is about 10,000-100,000.

***Note:**

a) Cell density directly affects mycoplasma elimination, and for 6 cm plates, it is recommended to control the cell number between 10,000-100,000.

b) Add the reagents in the order above, i.e., add the contaminated cell suspension to the medium containing the Myco-Clear Mycoplasma Removal Agent. The order of addition cannot be reversed.

4) Culture cells normally until the density reaches 80-90% (typically 5-8 days), at which point it can be subcultured with fresh normal cell culture medium.

***Note:** It is recommended to continuously observe to check the cytotoxic effects, and if clearly noticeable, the medium can be changed or diluted to stop the treatment. If the cells grow slowly and do not reach passage density after 5-8 days, stop the treatment by discarding the medium containing the Myco-Clear Mycoplasma Removal Agent and adding fresh normal cell culture medium.

2. Suspension cell treatment

1) Harvest contaminated cells and adjust the cell density to 6,000-60,000 cells/mL with fresh medium containing 10% fetal bovine serum.

2) Add 1.6 mL of PBS containing 0.125% trypsin, 5 mM EDTA to a 15 mL sterile centrifuge tube. Then add 200 µL of Myco-Clear Mycoplasma Removal Agent and mix well.

3) Add 1.6 mL of the cell suspension prepared in step 1 and mix. At this point, the number of cells is about 10,000-100,000.

***Note:** Add the reagents in the order above, i.e., add the contaminated cell suspension to the medium containing the Myco-Clear Mycoplasma Removal Agent. The order of addition cannot be reversed.

4) Place the centrifuge tubes on a shaker and incubate for 30 min at 37°C with slow shaking.

***Note:** The incubation time can be optimized to 15-20 min for some sensitive cells.

5) Centrifuge at 600 g for 10 min and discard the supernatant.

6) Resuspend cells in fresh medium containing 10% fetal bovine serum and then culture normally.

7) (Optional) To kill mycoplasma more completely, you can continue with the following steps after step 5.

8) Resuspend cells in 5 mL of fresh medium containing 5% fetal bovine serum, add 150 μL of Myco-Clear Mycoplasma Removal Agent, mix well, and transfer to a 6 cm plate for 3 days.

***Note:** It is recommended to continuously observe to check the cytotoxic effects, and if clearly noticeable, the medium can be changed or diluted to stop the treatment

9) Replace the medium with fresh normal medium containing 10% fetal bovine serum and continue the culture.

3. No enveloped virus treatment

Fresh or frozen viral solutions can be used, and viral titer does not influence the treatment.

1) Add 1 mL of serum-free medium to a sterile 1.5 mL centrifuge tube.

2) Add 100 μL of Myco-Clear Mycoplasma Removal Agent and mix well.

3) Add 125 μL of virus solution (serum concentration ≤8%) and mix by vortexing gently. Incubate for 2 h at room temperature. The volume of the mixed solution is approximately 1.2 mL at this point.

***Note:** During vortexing, make sure that the mixed solution thoroughly wets the inner wall of the centrifuge tube to avoid local mycoplasma contamination that is not treated.

4) After incubation, stop the treatment by diluting the mixed solution with fresh normal cell culture medium at the ratio of 1:9. For example, add 10.8 mL of fresh cell culture medium to 1.2 mL of the mixed solution. Or dilute the mixture 10-fold directly into the cells to be infected.

***Note:** The host cells need to be tested for mycoplasma contamination to ensure that the host cells are free of mycoplasma contamination.

4. Enveloped virus treatment

Fresh or frozen viral solutions can be used, and it is recommended that the viral titer should be higher than 10^6 $TCID_{50}$.

1) Add 4.4 mL of serum-free medium to a 15 mL sterile centrifuge tube.

2) Add 100 μL of Myco-Clear Mycoplasma Removal Agent and mix well.

3) Add 500 μL of virus solution (serum concentration ≤8%) and mix by vortexing gently. Incubate for 2 h at room temperature. The volume of the mixed solution is 5 mL.

***Note:** During vortexing, make sure that the mixed solution thoroughly wets the inner wall of the centrifuge tube to avoid local mycoplasma contamination that is not treated.

4) After incubation, stop the treatment by diluting the mixed solution with fresh normal cell culture medium at the ratio of 1:9. For example, add 45 mL of fresh cell culture medium to 5 mL of the mixed solution. Or dilute the mix 10-fold directly into the cells to be infected.

*Note:

a) For enveloped viruses, the mycoplasma removal process described above can be repeated several times to completely kill the mycoplasma.

b) The host cells need to be tested for mycoplasma contamination to ensure that the host cells are free of mycoplasma contamination.

5. **Detect the mycoplasma**

1) If using the PCR-based detection methods, treated cell should be subcultivated for 3-4 times before testing for mycoplasma. This is because when the mycoplasma membrane is ruptured, mycoplasma DNA is released into the culture medium, resulting in a false positive for mycoplasma nucleic acid detection.

*Note: You can choose the PCR Mycoplasma Detection Kit (Cat. No. K2821) or the Mycoplasma qPCR Detection Kit (Cat. No. K2822) to detect the mycoplasma.

2) If using the bioluminescence detection method, it can be detected after a certain period of culture in a normal cell culture medium.

Note

1. Cell density directly affects mycoplasma elimination, and for 6 cm plates, it is recommended to control the cell number between 10,000-100,000.
2. Before using this reagent, make sure that the cells are all trypsinized into single cells and do not form clumps. This is because this reagent requires direct contact with mycoplasma to work. Clumping of cells can be avoided by extending the trypsinization time.
3. High concentrations of fetal bovine serum also affect mycoplasma elimination. In the process of mycoplasma removal, it is recommended to use fetal bovine serum at a concentration of 5%.
4. The cell proliferation may be affected during the treatment. It is recommended to continuously observe to check the cytotoxic effects, and if clearly noticeable, the medium can be changed or diluted to stop the treatment.
5. Before mycoplasma elimination, the host cells need to be tested for mycoplasma contamination to ensure that the host cells are free of mycoplasma contamination.
6. The plasma membrane of the enveloped virus is similar to that of mycoplasma, so it also can bind to this reagent and be cleared. To ensure complete elimination of mycoplasma and the ability of the virus to infect, it is recommended that the starting viral titer should be higher than 10^6 $TCID_{50}$. There is no such titer requirement for non-enveloped viruses.
7. Although this reagent can almost completely eliminate mycoplasma, cells can still be contaminated again due to environmental, reagent, and handling factors. Regular testing for mycoplasma is recommended.

8. It is suggested to use the PCR Mycoplasma Detection Kit (Cat. No. K2821) or the Mycoplasma qPCR Detection Kit (Cat. No. K2822) to detect the mycoplasma.

9. For your safety and health, please wear lab coats and gloves during the experiment.

10. For research use only. Not to be used in clinical diagnostic or clinical trials.

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