Myco-Clear Mycoplasma Removal Agent Plus

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 Plus is a surfactant peptide extracted from the ferment of Bacillus subtilis, which can selectively bind to the mycoplasma membrane and change the permeability of the mycoplasma membrane, 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 2-5 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. This reagent is an upgraded version of Myco-Clear Mycoplasma Removal Agent (Cat. No. K2824), which is more convenient to use and has a better result for elimination.

Components and Storage

Components \ Size	100 μL	500 μL	2 mL	Storage
Myco-Clear Mycoplasma Removal Agent Plus	100 μL	500 μL	2 mL	4°C
Shipping: Blue ice	Shelf life: 1 year			

Protocol

1. **Preparation before the experiment:**

1) For cells, mix an appropriate amount of Myco-Clear Mycoplasma Removal Agent Plus and fresh normal cell culture medium at a ratio of 1:1000 to obtain mycoplasma removal solution. For example, dilute 10 μL of Myco-Clear Mycoplasma Removal Agent Plus in 10 mL of fresh cell culture medium.

2) For non-enveloped viruses, mix an appropriate amount of Myco-Clear Mycoplasma Removal Agent Plus and fresh serum-free medium at a ratio of 1:800 to obtain mycoplasma removal solution.

3) For enveloped viruses, mix an appropriate amount of Myco-Clear Mycoplasma Removal Agent Plus and fresh serum-free medium at a ratio of 1:4000 to obtain mycoplasma removal solution.

***Note:** Mycoplasma removal solution needs to be prepared and used freshly.

2. Adherent cell treatment

1) Day 1: Trypsinize the contaminated cells in an appropriate amount of mycoplasma removal solution and culture cells as usual.

***Note:**

a) Cell should reach more than 60% confluency on the second day.

b) This reagent requires direct contact with mycoplasma to work. Therefore, adherent cells need to be treated with mycoplasma removal solution before seeding.

2) Day 3: Change or passage with mycoplasma removal solution. If the cells reach passage density, passage is performed. If this is not achieved, replace with fresh mycoplasma removal solution and continue processing.

***Note:**

a) 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.

b) Mycoplasma can be removed after 1-2 treatments, depending on the severity of mycoplasma contamination.

3) After mycoplasma elimination, change with normal cell culture medium to culture cells.

3. Suspension cell treatment

1) Day 1: Collect contaminated cells by 1,000 g centrifugation for 5 min. Discard the supernatant, add an appropriate amount of mycoplasma removal solution to resuspend the cells, and culture cells as usual.

2) Day 3: Replace with the mycoplasma removal solution, or remove the mycoplasma removal solution directly.

***Note:**

a) 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.

b) Mycoplasma can be removed after 1-2 treatments, depending on the severity of mycoplasma contamination.

3) After mycoplasma elimination, change with normal cell culture medium to culture cells.

4. No enveloped virus elimination

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

1) Gently vortex and mix the virus solution and mycoplasma removal solution at a ratio of 1:10. For better elimination effect, there should be less fetal bovine serum in the virus solution.

***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.

2) Incubate for 2 h at room temperature.

3) 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 9 mL of fresh medium to 1 mL of the mix and mix well, or dilute the mix 10-fold directly into the cells to be infected.

***Note:** Before performing mycoplasma elimination, the host cells need to be tested for mycoplasma contamination to ensure that the host cells are free of mycoplasma contamination.

5. Enveloped virus elimination

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) Gently vortex and mix the virus solution and mycoplasma removal solution at a ratio of 1:10. For better elimination effect, there should be less fetal bovine serum in the virus solution.

***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.

1) Incubate for 30 min at room temperature.

2) 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 9 mL of fresh medium to 1 mL of the mix and mix well, 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) 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. 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. This reagent requires direct contact with mycoplasma to work. Therefore, adherent cells need to be treated with mycoplasma removal solution before seeding.
2. 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.
3. At the same time, it is necessary to optimize the amount of cells, and the density of adherent cells is generally required to be more than 60%; In a 6 cm plate, the number of suspension cells is more than 300,000. However, the number of cells should not be too large, which will affect the effect of elimination. It can be optimized according to specific experiments.
4. Before mycoplasma elimination, the host cells need to be tested for mycoplasma contamination to ensure that the host cells are free of mycoplasma contamination.
5. 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.
6. 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.
7. For your safety and health, please wear lab coats and gloves during the experiment.
8. For research use only. Not to be used in clinical diagnostic or clinical trials

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