



BioMycoX[®]

Mycoplasma Elimination Kit

Version:4.0

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Instruction Manual

Cat. No. E-01, E-02, E-03

Research Use Only. Not for Use in Diagnostic Procedures.

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1. Product Information

Introduction

BioMycoX[®] Mycoplasma Elimination Kit has been developed to rapidly and completely eliminate mycoplasma contamination from cell cultures. The potential impact of mycoplasma contamination in cell culture is a major concern in research, diagnostics, and the production of biological products. Mycoplasma contamination reduces the quantity and quality of the produced products, leading to inconsistency and a lack of reproducibility in experiments.

Current methods for inactivating or eliminating mycoplasma in cell cultures primarily rely on antibiotics. However, antibiotic treatments, in particular, require prolonged usage, making them prone to the development of resistance through continuous antibiotic exposure. Additionally, some antibiotics can alter the metabolism of eukaryotic cells, inducing cytotoxicity and potentially impacting cell cultures.

The BioMycoX[®] Mycoplasma Elimination Kit effectively removes mycoplasma contamination without the risks associated with antibiotics, such as side effects and the development of resistance. This kit is easy to use by simply adding it to the culture medium during cell passaging, making it an excellent solution for eliminating mycoplasma contamination (*Mycoplasma sp.*, *Acholeplasma sp.*, *Ureaplasma sp.*, etc.) from contaminated cells.

2. Contents and Storage

Materials Provided

Label	Cap	E-01	E-02	E-03
BioMycoX [®] Mycoplasma Elimination Reagent I	Green	200 µl	400 µl	600 µl
BioMycoX [®] Mycoplasma Elimination Reagent II	Blue	200 µl	400 µl	600 µl
Cell Strainer	-	2 ea	4 ea	6 ea

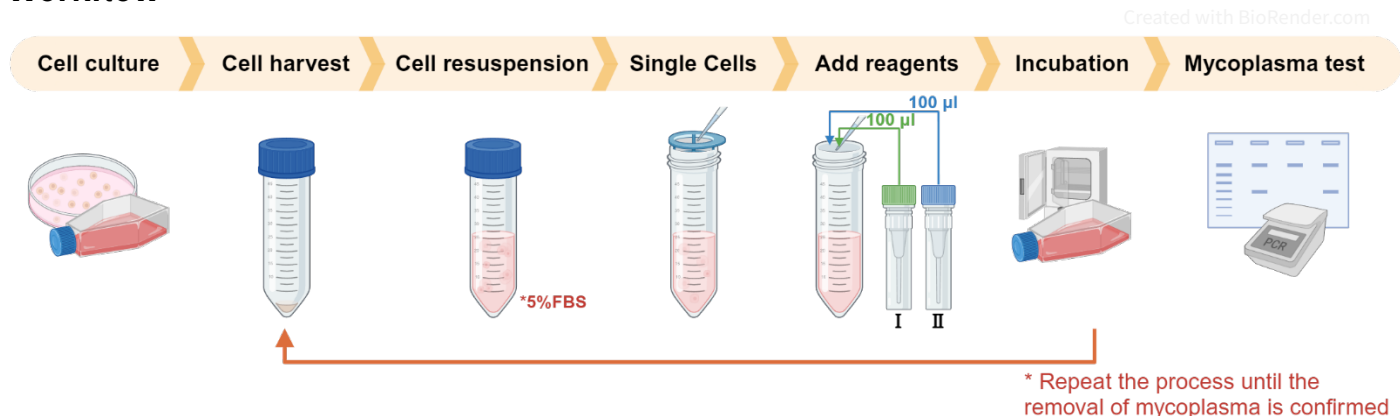
Storage

Upon receipt, store at 2~8°C.

Please check the label on the product for details.

3. Test protocol

Workflow



Protocol

- 1) Prepare cells contaminated with mycoplasma.
- 2) Remove the medium from cultured cells (80-90% confluent) and dissociate the cells using an appropriate method (e.g., trypsinization).
- 3) Add an appropriate medium and harvest the cells using centrifugation.
- 4) Resuspend the harvested cell pellet in 10 ml of growth medium containing 5% FBS*.
**It is crucial to use a growth medium with 5% FBS, as concentrations above 5% may reduce the efficacy of mycoplasma removal.*
- 5) Adjust the cell suspension to a cell density of $5 \times 10^5 \sim 5 \times 10^6$ cells**.
***This is the recommended cell number and should be adjusted based on cell type and characteristics.*
- 6) Strain the cell suspension through the cell strainer provided in the kit to generate single cells.
- 7) Add 100 μ l of BioMycoX® Reagent I and 100 μ l of BioMycoX® Reagent II to the 10ml cell suspension and mix carefully.
- 8) Transfer the cell suspension to a culture vessel as a 100mm culture dish or T75 culture flask and incubate for 2~3 days.
- 9) Proceed with different steps based on the status of cell growth.
 - 9-1) In case of reaching 80-90% confluence, repeat the elimination reagent treatment.
(return to protocol step 2).
 - 9-2) In case of slow cell growth, proceed to step 10).
- 10) Prepare a new growth medium containing 10ml with 5% FBS, to which 100 μ l each of BioMycoX® Reagent I and BioMycoX® Reagent II is added.
- 11) Remove the previous medium and replace it with the prepared new medium in the culture vessel. Incubate for 2-3 days.

Once mycoplasma removal is initiated, it is essential to continuously treat with elimination reagents until mycoplasma is completely eliminated. Therefore, repeat the above protocol iteratively based on the severity of mycoplasma contamination. The table below shows the number of repetitions for each product.

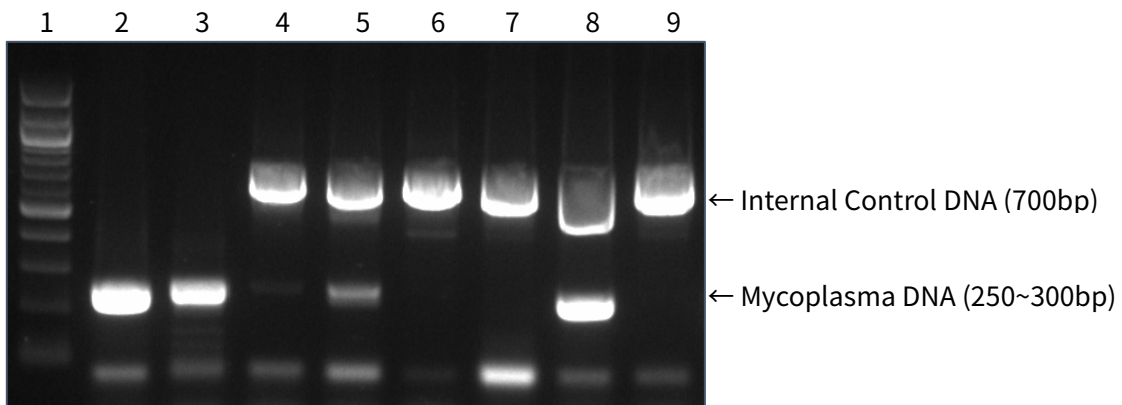
Cat. No.	Number of elimination reagent treatment
E-01	2
E-02	4
E-03	6

12) Confirm the success of the treatment by testing with the BioMycoX® Mycoplasma PCR Detection Kit.

4. Results

Result of Mycoplasma Elimination (PCR)

Tested with BioMycoX® Mycoplasma PCR Detection Kit (Cat. No. D-25, CellSafe)



Lane 1 : 100bp DNA ladder

Lane 2 : Before treatment with elimination reagent

Lane 3 : After treatment with elimination reagent once

Lane 4 : After treatment with elimination reagent twice

Lane 5 : Culture after treatment with elimination reagent twice

Lane 6 : After treatment with elimination reagent 4 times

Lane 7 : Culture after treatment with elimination reagent 4 times

Lane 8 : Positive control

Lane 9 : Negative control