

## Mycoplasma Elimination Kit Product Manual

### ▶ Product Information

| Catalog Number | Component | Specification |
|----------------|-----------|---------------|
| EDME-01        | MycoE-A   | 1 mL          |
|                | MycoE-B   | 1 mL          |
| EDME-02        | MycoE-A   | 5 mL          |
|                | MycoE-B   | 5 mL          |

### ▶ Product Overview

Mycoplasma, the smallest and simplest prokaryotes, can alter cellular DNA, RNA, and protein expression, leading to slow cell growth and morphological changes, which severely impact experimental results. If cells are contaminated with Mycoplasma, it is recommended to discard them after autoclaving. However, for valuable contaminated cells, Mycoplasma elimination reagents can be used.

The key components of this product are two different antibiotics, both of which inhibit protein synthesis. Alternating the use of these antibiotics effectively reduces the development of resistance. This product suppresses and eliminates various Mycoplasma species, including *Mycoplasma orale*, *M. arginini*, *M. hyorhinitis*, and *Acholeplasma laidlawii*, without affecting cell health, thereby saving valuable contaminated cells.

### ▶ Storage

Shipped with ice packs; store at -20° C. Shelf life: 12 months. Store in a dark place if not used for extended periods.

### ▶ Instructions for Use

1. Prepare a complete medium containing 20% FBS and use it to culture cells during Mycoplasma

elimination;

2. Subculture cells into a T25 flask, maintaining ~50% confluency;
3. Once cells adhere and appear normal, add 20  $\mu$  L of MycoE-A directly to the cultured cells;

**Note: For a 5 mL culture system, use 4  $\mu$  L of MycoE-A per 1 mL of medium when changing culture vessels.**

**Note: Add the reagent directly to the cells rather than preparing it in the medium beforehand.**

4. Replace the medium or subculture within 48 hours, continuing to add Mycoplasma elimination reagents;
5. After 4 days of culturing, replace MycoE-A with an equivalent amount (20  $\mu$  L) of MycoE-B and continue culturing for 3 days;
6. Repeat steps 3 through 5 once more to complete Mycoplasma elimination;
7. Test for Mycoplasma contamination using DNA staining (DAPI) or PCR methods.

## ► Frequently Asked Questions

### 1. Cell death or poor cell condition after Mycoplasma elimination

For most cell lines, Mycoplasma elimination does not affect cell health. However, for sensitive cells, it is recommended to back up the cells before treatment. If cell death or poor conditions occur, reduce the working concentration by half and extend the treatment cycle to 28 days.

### 2. Residual Mycoplasma detected after treatment

In most cases, Mycoplasma should be completely eliminated after treatment. If residual contamination persists, increase the working concentration by 50% and repeat the treatment for another cycle.

### 3. Can MycoE-A and MycoE-B be used simultaneously?

No, simultaneous use of both reagents places excessive stress on cells and increases the likelihood of resistance development. Use the reagents sequentially according to the instructions.

### 4. Can this reagent be used alongside conventional antibiotics like penicillin, streptomycin, amphotericin, or gentamicin?

Since this reagent already provides antibiotic functionality, additional antibiotics are not recommended during the treatment to avoid adding unnecessary stress to the cells.



## ► Precautions

- (1) This product is for laboratory use only and intended for research purposes. Please strictly follow relevant laws, regulations, and ethical requirements. The company is not responsible for any consequences arising from misuse.
- (2) Ensure proper transportation, storage, and use of the reagent. Avoid repeated freeze-thaw cycles unless necessary. The company is not responsible for experimental failure caused by improper storage or handling.

