

# NutriCulture Mycoplasma Elimination Kit

Cat No: MEK25

**Shipping** : Ship with blue ice.  
**Storage** : Store at -20°C.

## General Information

Contamination of cell cultures with bacteria, fungi, and yeasts represents a major problem in cell culture. Although these microorganisms can be easily detected during routine cell culture applications via changes in the turbidity of the culture and visualization under the inverted microscope, a class of bacteria regularly escapes detection. These bacteria belonging to the class of Mollicutes, are commonly known as mycoplasma. Mycoplasma may survive undetected in cell cultures for a long time without any visible effect on the culture.

*NutriCulture* Mycoplasma Elimination Kit is used for removal of mycoplasma from infected cell cultures with no cytotoxic side effects observed. *NutriCulture* Mycoplasma Elimination Kit can effectively eliminate mycoplasma strains, that are responsible for more than 85% of the contaminations in animal cell cultures (*Acholeplasma laidlawii*, *Mycoplasma arginini*, *Mycoplasma hyorhina*, and *Mycoplasma orale*), from experimentally contaminated and chronically infected cell lines.

*NutriCulture* Mycoplasma Elimination Kit is comprised of two antibiotics, MEK1 and MEK2, which targets protein synthesis.

## Preparation of antibiotic stock solutions

Dissolve MEK1 and MEK2 antibiotics in 2 mL of sterile PBS or sterile ddH<sub>2</sub>O to obtain stock solutions of 250x. Mix well and aliquot antibiotic stock solutions to prevent repeated freeze thaw cycles. Stock solutions can be stored at -15 to -25°C for at least 6 months. Antibiotics as lyophilized can be stored at -15 to -25°C for at least 12 months.

## Protocol for elimination of mycoplasma from the cell culture

**1.** Seed cells in T25 or T75 flasks in 5- or 10-ml appropriate medium, respectively. Incubate cells at 37°C in a humidified CO<sub>2</sub> incubator for 16-24 hours to achieve around 50-60% confluency.

**2.** Discard the medium, add fresh medium containing 1x MEK1 and **incubate for 3 days**. For example, for 5 ml cell culture medium add 20 µl of MEK1.

**Important Note:** *1x MEK1 does not affect the growth of most cells. For sensitive cell lines, lower concentrations may be used. It is not recommended to use MEK1 together with other antibiotics, therefore, use fresh medium supplemented with fetal bovine serum with no additional antibiotics such as penicillin or streptomycin.*

**3.** Discard the medium, add fresh medium containing 1x MEK2 and **incubate for 4 days**. For example, for 5 ml cell culture medium add 20 µl of MEK1.

**Important Note:** *1x MEK2 does not affect the growth of most cells. For sensitive cell lines, lower concentrations may be used. It is not recommended to use MEK2 together with other antibiotics, therefore, use fresh medium supplemented with fetal bovine serum with no additional antibiotics such as penicillin or streptomycin.*

*Please use the MEK-1 and MEK-2, respectively. Do not use them together.*

**4.** Repeat steps 2 and 3 for up to 4 consecutive weeks in total.

**Important Note:** *For highly contaminated cell cultures or resistant strains, both MEK1 and MEK2 antibiotics can be applied up to 2x concentration for up to 8 consecutive weeks to get mycoplasma negative cells.*

**5.** Check positivity of cells with *NutriCulture* Mycoplasma Detection Kit (Cat No: MDK50) at the end of each cycle.

6. After observing that the cells are negative for mycoplasma, culture cells with no antibiotics for at least 2 weeks and test again for mycoplasma to ensure that cells are mycoplasma negative.

### **Additional Notes**

Use always filtered tips when culturing cells.

Strictly bind to aseptic cell culture conditions when working with cells.

Note that the most important source of mycoplasma contamination is human. Therefore, a clean laboratory coat and a face mask are recommended to be used when working with cells.

Every incoming cell culture should be kept in quarantine until mycoplasma detection assays are completed and the infection status is determined.