Plasmocin™ Treatment

For the treatment of mycoplasma contamination of cell culture

Catalog # ant-mpt

For research use only

Version # 12I12-MM

PRODUCT INFORMATION

Content:

- 50 mg Plasmocin™ Treatment, provided in 2 x 1 ml tubes at a concentration of 25 mg/ml.

Shipping and Storage:

- Plasmocin™ Treatment is shipped at room temperature and should be stored at 4°C or -20°C.
- Plasmocin™ Treatment is stable 2 weeks at room temperature, 6 months at 4°C, and 2 years at -20°C. Avoid repeated freeze-thaw cycles.

Note: *Presence of crystals does not alter properties of the product. Vortex the tube until the crystals disappear.*

Quality Control:

Activity of Plasmocin™ Treatment is rigorously controlled by physicochemical and microbiological assays.

GENERAL PRODUCT USE

Plasmocin™ Treatment is used to cure cell lines infected by mycoplasma.

BACKGROUND

Mycoplasma contamination of cultured cells is a major problem in both basic research and industrial production. Up to 87% of cell lines may be contaminated by mycoplasma[1,2]. Unlike bacterial or fungal contaminations, mycoplasmal contaminations cannot be detected by visual inspection and may not noticeably affect cell culture growth rates. However, mycoplasma infection has been shown to alter DNA, RNA and protein synthesis, introduce chromosomal aberrations and cause alterations or modifications of host cell plasma membrane antigens.

DESCRIPTION

Plasmocin™ is a well-established anti-mycoplasma reagent. It contains two bactericidal components. The first component acts on the protein synthesis machinery by interfering with ribosome translation, and the other acts on DNA replication. These two specific and separate targets are found in mycoplasma and many bacteria, but are completely absent in eukaryotic cells. In contrast to other anti-mycoplasma compounds, Plasmocin™ is active on both free mycoplasmas and intracellular forms. This advantage is conferred by one component of Plasmocin™ which is actively transported into mammalian cells.

Plasmocin™ is active at low concentrations on a broad range of gram positive and gram negative bacteria otherwise resistant to the mixture of streptomycin and penicillin antibiotics commonly used in cell cultures.

In all animal cell lines tested to date, even at five times the working concentration, no apparent adverse effect on cellular metabolism is observed. At high concentrations of Plasmocin™, slowdown of cell growth rate may be observed. This slowing down is mainly due to the inhibition of mitochondrial respiration by Plasmocin™. However when Plasmocin™ is removed from culture medium, cells return rapidly to their normal growth rate. The anti-mycoplasma activity of Plasmocin™ is unaltered in cell culture medium containing up to 20% serum.

Many cell lines infected by mycoplasmas have been successfully treated with Plasmocin™, including hybridomas, lymphocytes, epithelial cells[3], murine embryonic stem cells, and retrovirus packaging cells. It has been shown that treatment with Plasmocin™ restores cellular responses following mycoplasma clearance[4, 5].

1. Lincoln CK. & Gabridge MG., 1998. Cell culture contamination: sources, consequences, prevention, and elimination. Methods Cell Biol. 57:49-65. **2. Uphoff CC. & Drexler HG., 2002. C**omparative PCR analysis for detection of mycoplasma infections in continuous cell lines. In Vitro Cell Dev Biol Anim. 38:79-85. **3. Kazemiha VM. *et al*., 2011.** Efficiency of PlasmocinTM on various mammalian cell lines infected by mollicutes in comparison with commonly used antibiotics in cell culture: a local experience. Cytotech. [Epub ahead of print]. **4. Zakharova E. *et al*., 2010.** Mycoplasma suppression of THP-1 cell TLR responses is corrected with antibiotics. PLoS One. 25;5(3):e9900. **5. Jetté L. *et al*., 2008.** Resistance of colorectal cancer cells to 5-FUdR and 5-FU caused by mycoplasma infection. Anticancer Res. 28: 2175 - 2180.

METHOD

Treatment of Mycoplasma Infected Cell Cultures:

Typically, Plasmocin™ Treatment is used for 2 weeks at 25 µg/ml which represents a 1:1000 dilution of the 25 mg/ml stock solution (100 µl Plasmocin™ Treatment in 100 ml culture medium). Plasmocin™ Treatment can be used in combination with penicillin and streptomycin.

1. Split an actively dividing culture of cells into medium containing 25 µg/ml of Plasmocin™ Treatment.
2. Remove and replace with fresh Plasmocin™ Treatment containing medium every 3-4 days for 2 weeks.

Note: If 25 µg/ml of Plasmocin™ is toxic for your cells, reduce the concentration of Plasmocin™ to 12.5 µg/ml and treat for an additional week.

3. Test for the presence of mycoplasma by a conventional PCR method or using a cell based assay. If mycoplasmas are still present following treatment, see troubleshooting overleaf.

Note: InvivoGen provides PlasmoTest™ a simple, rapid and reliable assay for the visual detection of mycoplasma contamination. This is the first assay to utilize cells to signal the presence of mycoplasma.

4. For the maintenance of a mycoplasma free culture, use Plasmocin™ Prophylactic at a concentration of 5 µg/ml.

TECHNICAL SUPPORT
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RESISTANCE TO PLASMOCIN™

In repeated experiments aimed to determine the mutation rate of *Mycoplasma hominis*, *Mycoplasma bovis* and *Acholeplasma vituli* to Plasmocin™, no resistance in liquid cultures has ever been identified, indicating a possible mutation rate lower than 10^{-9}. Therefore, development of resistant mycoplasma strains is highly unlikely.

TROUBLESHOOTING

Some cells may be sensitive to Plasmocin™ Treatment. If 25 µg/ml of Plasmocin™ is toxic for your cells, we recommend that you reduce the concentration of Plasmocin™ to 12.5 µg/ml and increase the duration of the treatment.

Following treatment with Plasmocin™, mycoplasmas should be eliminated or reduced. If mycoplasma contamination is reduced but still present, treat your cells with 37.5 µg/ml of Plasmocin™ for a further week.

However, if there is no reduction in the mycoplasma contamination following treatment with Plasmocin™, the mycoplasma infecting your cells may be resistant to Plasmocin™. In this instance, we recommend using Plasmocure™, an alternative mycoplasma removal agent. Plasmocure™ combines two antibiotics that act through different mechanisms of action than those in Plasmocin™. A two week treatment with Plasmocure™ is typically sufficient to completely eliminate the mycoplasmas. A moderate toxicity can be observed during the course of the treatment but full recovery of the cell line is expected once mycoplasmas are eliminated.

RELATED PRODUCTS

Product	Catalog Code
PlasmoTest™	rep-pt2
Plasmocin™ Prophylactic	ant-mpp
Plasmocure™	ant-pc

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