Plasmocin™ Treatment

For the elimination of mycoplasma contamination in cell cultures

Catalog # ant-mpt
http://www.invivogen.com/plasmocin

For research use only. Not for human or veterinary use.

Version # 16F09-MM

PRODUCT INFORMATION

Content

Plasmocin™ Treatment is supplied as a cell culture tested, sterile filtered yellow solution at 25 mg/ml.

- **ant-mpt:** 2 x 1 ml (50 mg)

One 1 ml vial is sufficient for 660 ml to 2 liters of culture.

Shipping and Storage

- Plasmocin™ Treatment is shipped at room temperature. Upon receipt, it can be stored at 4 °C for 1 month or at -20 °C for long-term storage. Avoid repeated freeze-thaw cycles.
- The expiry date is specified on the product label.

Notes:

- *During storage a crystalline precipitate may form. If this occurs, vortex the product until the crystalline precipitate disappears. The formation of a crystalline precipitate does not affect the activity of the product.*
- *Product is stable for 2 weeks at room temperature.*

QUALITY CONTROL

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation:

- Endotoxin level: < 2 EU/mg
- Physicochemical characterization (pH, appearance)
- Cell culture tested: potency validated on bacterial reference strains

BACKGROUND

Mycoplasma contamination is a significant problem for mammalian cell culture. Reports estimate mycoplasma contamination in up to 35% of all cell cultures[1,2]. Unlike bacterial or fungal contaminations, mycoplasma 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 highly cited broad-spectrum anti-mycoplasma reagent. Cell cultures contaminated with mycoplasmas, such as *M. arginini*, *M. fermentans*, *M. laidlawii*, and *M. hyorhinis* can be efficiently cured by Plasmocin™ treatment. In contrast to other anti-mycoplasma compounds, Plasmocin™ is active on both extracellular mycoplasmas and intracellular forms. This advantage is conferred by one component of Plasmocin™ that is actively transported into mammalian cells.

In addition, Plasmocin™ is active at low concentrations on a broad range of Gram-positive bacteria, such as *Staphylococcus* species, and Gram-negative bacteria, such as *E. coli*, *Enterobacter*, *Pseudomonas*, and *Alcaligenes*.

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].

The cytotoxicity of Plasmocin™ is low, however a slowdown of cell growth may be observed. At the end of the treatment, when Plasmocin™ is removed from the culture medium, the cells return rapidly to their normal growth rate. Plasmocin™ may be added to media containing penicillin and streptomycin (Pen-Strep). Its anti-mycoplasma activity is unaltered in cell culture medium containing up to 20% serum.

COMPOSITION

Plasmocin™ 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 absent in eukaryotic cells.

METHOD

The working concentration of Plasmocin™ Treatment varies from 12.5 to 37.5 µg/ml. It can be added directly to the bottle of culture medium or to the flask containing the cells. To determine the optimal concentration for your cells, we recommend to test in parallel the 3 different concentrations shown in the table below. Refer to this table to determine the volume of Plasmocin™ needed.

Note: For small volumes, intermediary dilutions may be prepared with sterile culture medium.

Plasmocin™ final concentration	T25 with 5 ml medium	T75 with 15 ml medium	500 ml bottle
12.5 µg/ml	2.5 µl	7.5 µl	250 µl
25 µg/ml	5 µl	15 µl	500 µl
37.5 µg/ml	7.5 µl	22.5 µl	750 µl

1. Remove medium from contaminated cells and rinse twice with phosphate buffered saline (PBS).
2. Split an actively dividing culture of cells into medium containing Plasmocin™. Ensure your cells are in the exponential growth phase by passing them at an appropriate dilution (e.g. 1:10).
3. Remove and replace with fresh Plasmocin™ Treatment containing medium every 3-4 days for 2 weeks.
4. Confirm the elimination of mycoplasmas by using a mycoplasma detection kit such as PlasmoTest™, a cell-based colorimetric assay.

Note: If mycoplasma elimination is not completed after a 2-week treatment, see the troubleshooting section on the next page.

5. For the maintenance of a mycoplasma-free culture, use Plasmocin™ Prophylactic (see Related Products on the next page).

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

TROUBLESHOOTING

Following a 2-week treatment with Plasmocin™, mycoplasmas should be eliminated. If mycoplasma contamination is reduced but still present, treat your cells with 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 2-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.

DEVELOPMENT OF PLASMOCIN™-RESISTANCE

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 resistance in these reference mycoplasma strains is highly unlikely.

REFERENCES

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.** Comparative 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 Plasmocin™ on various mammalian cell lines infected by mollicutes in comparison with commonly used antibiotics in cell culture: a local experience. Cytotech. 63(6):609-20. **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: 2175-80.

RELATED PRODUCTS

Product	Description	Cat. Code
Normocin™	Antimicrobial agent	ant-nr-1
Normocure™	Antibacterial agent	ant-noc
Plasmocin™ Prophylactic	Anti-mycoplasmal agent	ant-mpp
Plasmocure™	Mycoplasma removal agent	ant-pc
PlasmoTest™	Mycoplasma detection kit	rep-pt1
Primocin™	Antimicrobial for primary cells	ant-pm-1

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InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com