Plasmocin™ Treatment
For the elimination of mycoplasma contamination in cell cultures
Catalog code: ant-mpt-1, ant-mpt
http://www.invivogen.com/plasmocin

For research use only. Not for human or veterinary use.
Version 18D13-MM

PRODUCT INFORMATION
Content
Plasmocin™ Treatment is supplied as a cell culture tested, sterile filtered yellow solution at 25 mg/ml.
- ant-mpt-1: 1 ml (25 mg)
- 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. 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, murine embryonic stem cells, and retrovirus packaging cells. It has been shown that treatment with Plasmocin™ restores cellular responses following mycoplasma clearance.

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 specific targets are absent in eukaryotic cells ensuring low cytotoxicity.

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.

<table>
<thead>
<tr>
<th>Plasmocin™ Final concentration</th>
<th>T25 with 5 ml medium</th>
<th>T75 with 15 ml medium</th>
<th>500 ml bottle</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5 µg/ml</td>
<td>2.5 µl</td>
<td>7.5 µl</td>
<td>250 µl</td>
</tr>
<tr>
<td>25 µg/ml</td>
<td>5 µl</td>
<td>15 µl</td>
<td>500 µl</td>
</tr>
<tr>
<td>37.5 µg/ml</td>
<td>7.5 µl</td>
<td>22.5 µl</td>
<td>750 µl</td>
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</tbody>
</table>

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
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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

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>ant-nr-1</td>
</tr>
<tr>
<td>Normocure™</td>
<td>Antibacterial agent</td>
<td>ant-noc</td>
</tr>
<tr>
<td>Plasmocin™ Prophylactic</td>
<td>Anti-mycoplasma agent</td>
<td>ant-mpp</td>
</tr>
<tr>
<td>Plasmocure™</td>
<td>Mycoplasma removal agent</td>
<td>ant-pc</td>
</tr>
<tr>
<td>PlasmoTest™</td>
<td>Mycoplasma detection kit</td>
<td>rep-pt1</td>
</tr>
<tr>
<td>Primocin™</td>
<td>Antimicrobial for primary cells</td>
<td>ant-pm-1</td>
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