E30. How to Eliminate Mycoplasma Using Plasmocin™ (Invivogen)

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Plasmocin™ Treatment: To eliminate mycoplasmas use Plasmocin™ (**Invivogen Cat#: ant-mpt**) at **25 µg/ml** for two weeks in the infected culture.

Plasmocin™ Prophylactic: To prevent mycoplasma contamination, use Plasmocin™ (ant-mpp) at 2.5 - 5 µg/ml on a regular basis in cell culture.

Plasmocin™ is provided as a yellow solution at **25 mg/ml** (Plasmocin™ Treatment).

Description: Plasmocin™ is a well-established antimycoplasma reagent. It contains two bactericidal components strongly active against mycoplasmas that allow their elimination in **only 2 weeks**. The first component acts on the protein synthesis machinery while the second acts on the DNA replication. These two specific and separate targets are found only in mycoplasmas and many other bacteria and 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. It ensures that following treatment with Plasmocin™ a cell culture is not reinfected by mycoplasmas released from intracellular compartments of infected cells.

In all animal cell lines tested to date, even at five times the working concentration, no apparent adverse effect on cellular metabolism is observed. No resistance in liquid cultures of mycoplasmas has ever been identified in repeated experiments attempting to measure the mutation rate. Therefore, development of resistant mycoplasma strains is virtually eliminated. Plasmocin™ is also active at low concentrations on a broad range of Gram positive and Gram negative bacteria that are otherwise resistant to the mixture of streptomycin and penicillin, and exhibits no toxicity in eukaryotic cells. Many cell lines infected by mycoplasmas have been treated with Plasmocin™, including embryonic stem cells, hybridomas and retrovirus packaging cells.

Treatment of Mycoplasma Infected Cell Cultures

Plasmocin™ treatment (ant-mpt) requires little hands-on manipulation and is completed in only two weeks. Typically, **Plasmocin™** is used at **25µg/ml** which represents a 1:1000 dilution of the 25 mg/ml stock solution. Working concentration of **Plasmocin™** ranges from 12.5 to 37.5 µg/ml.

1. Split an actively dividing culture of cells into medium containing 12-25µg/ml of **Plasmocin™**.
2. Remove and replace with fresh **Plasmocin™** containing medium every 3-4 days for 2 weeks.
3. For maintenance of a Mycoplasma free culture, continue the use of **Plasmocin™** at a concentration of 5µg/ml.

Note: *If Mycoplasma elimination is not completed after a two-week treatment, you may continue the treatment for an additional week and/or increase the concentration to 37.5 µg/ml.*

Maintenance or prophylactic use against Mycoplasma infections

To prevent Mycoplasma and related cell wall-less bacteria contaminations of cell cultures that have been previously tested to be contamination-free, use **Plasmocin™** prevention (ant-mpp) at a concentration of **5µg/ml**.