

TOKU-E Protocol: De-Plasma™

(For primary cell lines and prophylactic use)



PRODUCT INFORMATION

Product name	De-Plasma™
Item #	D022
Format	25 mg/vial
Storage temp.	4°C
Shelf life	1 year

General Application

De-Plasma™ is designed to eliminate *Mycoplasma*™ contamination from cultured cell lines. It can also be used prophylactically to protect cultured cells from future *Mycoplasma* contamination.

Description

De-Plasma is an antibiotic cocktail comprised of one macrolide, one quinolone, and one tetracycline compound in a single vial. This three-compound mixture offers lower cytotoxicity and higher potency when compared with other products on the market. It is effective against all known *Mycoplasma* species and related non cell-walled prokaryotes.

Mode of Action

The modes of action for each of the antibiotics in De-Plasma are different. Tetracyclines and macrolides block protein synthesis by interfering with translation at the prokaryotic ribosome. Quinolones inhibit bacterial DNA gyrase and topoisomerase IV, blocking DNA replication. Eukaryotic cells do not contain DNA gyrase or topoisomerase IV and are therefore unaffected by De-Plasma treatment.

Method

De-Plasma can be added directly to the bottle of culture medium or to the flask containing the cells. It is recommended that a 1000x stock solution (or 5 mg/ml) be made of De-Plasma. To make the stock solution, add 1.0 ml water to one 25 mg vial, shake it, and transfer to a >10 ml container and then add another 4.0 ml water to make a final 5 ml stock solution of 5 mg/ml. The solution can be stored at 4°C for up to 3 months.

Working conc: 5 µg/ml

Decontamination period: 4 passages (~2-3 weeks; duration dependent on the growth of cells)

Conc. for maintenance of Mycoplasma-free culture: 2.5 µg/ml.

Caution

In order to maximize the effectiveness of De-Plasma, you should:

- Increase the FBS concentration 10% higher to enhance the cell growth.
- Mix the fresh cultures thoroughly with De-Plasma.
- Break the clumps and clusters of the cell lines.
- Keep cells' densities 5% to 10% higher than usual.
- Observe the cells under inverted microscope daily.

Protocol- Primary cell lines

1. Split an actively dividing culture of cells into media containing 5 µg/ml of De-Plasma.
2. Remove and replace with fresh De-Plasma-containing medium (5 µg/ml) every passage for 3 passages. (For some slow-growing cells, it is recommended to replace the media every ~2-3 days).
3. Confirm the elimination of *Mycoplasma* by using TOKU-E's PCR Mycoplasma Detection Kit.
4. If *Mycoplasma* has not been eliminated after 3 passages of treatment with De-Plasma, then treat with De-Plasma at 40 µg/ml for one additional passage.
5. Check for the elimination of *Mycoplasma* by using TOKU-E's PCR Mycoplasma Detection Kit.
For maintenance of Mycoplasma-free cultures, use De-Plasma at a concentration of 2.5 µg/ml.

Protocol- Prophylactic use

1. Split an actively dividing culture of cells into media containing 2.4 µg/ml of De-Plasma.
2. Confirm that cells grow normally in presence of De-Plasma.
3. For maintenance of *Mycoplasma*-free culture, continue using De-Plasma at a concentration of 2.5 µg/ml.
4. Check for the elimination of *Mycoplasma* by using TOKU-E's PCR Mycoplasma Detection Kit (Item # M034).