

## Treatment of Mycoplasma-infected Cells with Antibiotics

*Myco-1 (A5222), Myco-2 (A5233) and Myco-3 (A5240)*

---

### Description

The contamination of cells with mycoplasma is a very common problem, even though it often goes unnoticed since no cloudiness appears in the cell culture. Nevertheless the contamination often causes biochemical changes as well as changes in the immunological properties of the cells. Since mycoplasma infected cells cannot always be discarded, many complicated methods have been suggested for the elimination of the mycoplasma.

AppliChem is now offering a combination of antibiotics, which have been shown to be effective in the elimination of mycoplasma species that account for 90 % of the contamination found in cell culture. When used according to the following instructions, no cytotoxic effects will occur.

Store Myco solutions at -20°C. Several freeze / thawing cycles will not affect the quality.

### Myco-1 (A5222) and Myco-2 (A5233)

Myco-1 is based on the antibiotic Tiamulin, which is produced by the fungus *Pleurotus mutilus*. Myco-2 is based on Minocycline, a Tetracycline derivative. Myco-1 (A5222) and Myco-2 are generally used sequentially in combination. Embryonic stem cells (ES cells) were successfully treated with the two reagents. No cytotoxic effects were observed.

#### Instructions for use:

- 1.) Do not use the two solutions together, rather sequentially!
- 2.) Add 1 ml Myco-1 to 100 ml medium, and maintain the contaminated cells in this mixture for 4 days. Any fresh medium added should also contain Myco-1.
- 3.) After 4 days, add 1 ml Myco-2 to 100 ml fresh medium, and maintain the cells in this second mixture for 3 days.
- 4.) The above, together, are considered as one treatment cycle. it may be necessary to repeat this cycle 2-3 times.
- 5.) During the process, the cells can be tested for mycoplasma contamination, and results can then be used to shorten the process when possible.

### Myco-3 (A5240)

Myco-3 is based on the Ciprofloxacin antibiotic, which is a member of the fluoroquinolone group. Many mycoplasma species have been found to be sensitive to Myco-3, including *A. laidlawii*, *M. orale*, *M. hyorhinis*, *M. fermentans* and *M. arginini*. These species are responsible for most of the contamination in cell culture. At the concentrations recommended for use (~1 µg/ml), no cytotoxic effects have been found, and the treatment is quite easy to perform. The pH-value of the solution is 4 - 5. When added to medium the pH change is negligible.

#### Instructions for use:

- 1.) Add 1 ml Myco-3 to 100 ml medium.
- 2.) Continue the treatment for a total of 14 days, while changing the medium (containing Myco-3) every 2-3 days.
- 3.) Retain the cells in the growth medium for additional 14 days before re-testing for mycoplasma. Sometimes the treatment reduces the mycoplasma concentration without eliminating them and they will grow again after the antibiotic treatment. If this is the case, treat another time with the antibiotics Myco-1 and Myco-2.

### Literature

Schmitt, K. et al. (1988) *J. Immunol. Methods* **109**, 17-25