Mynox® & Mycoplasma-Off®
Frequently Asked Questions

Why do we note 'storage at 4-8°C' instead of room temperature, when shipping is performed at room temperature?

It is absolutely practicable to ship Mynox® at room temperature for the following reasons:
Mynox® is sterilized by autoclaving at 125 °C for 20 minutes and is stable at room temperature for at least 6 months even at higher temperatures. Anyhow the shelf life time of the product is much longer and sometimes customers use products even after the shelf life time has expired. To provide a product in good condition even for these circumstances we recommend long term storage as indicated on the label at +2 to +8°C (Mynox® is stable at these temperatures for at least 18 months). On the attached quality certificate you can find the information that shipment is regularly performed at room temperature to reduce additional costs for the end customer.

Mycoplasma is still detectable after treatment of cells with Mynox®.

Following the Mynox® treatment cells should be examined for remaining mycoplasma contamination after 4 passages when a sufficiently high cell density exists. Mynox® lyses the mycoplasma as an eradication mechanism. Thus, free mycoplasma DNA remains in the supernatant after treatment. With continuous cultivation and adequate cell density extracellular DNAses will hydrolyze free DNA.
Different factors might interfere with the efficiency of Mynox®.
A crucial factor is the FCS concentration. FCS contains cholesterol and other target molecules for Mynox®. Hence, it is pre-requisite to avoid higher FCS concentrations in the media than suggested (final concentration of FCS must be 5 %). Another crucial aspect for efficient mycoplasma elimination is the cell concentration. If elimination of mycoplasma did not occur with the first treatment we suggest to lower the cell concentration and/or to increase the incubation time with Mynox®.
Prior to the treatment with Mynox® make sure that you don't have cell clumps in your suspension. Extended trypsination will help to avoid the formation of cell clumps clearly.
In case of adherent cells it is highly recommended to use Petri dishes for the treatment. This will ensure that the cell suspension is not exposed to aerosols which could be produced when pipetting of the the cell suspension onto the Mynox® suspension is performed. The aerosols could stick to the surface of the vessel not being exposed to Mynox®. These contaminated aerosols could re-contaminate the cell culture later on. Therefore, it is very important to transfer the cells directly into the Mynox® suspension and not vice versa.
If the mycoplasma titer at the beginning of a treatment is extremely high it might be necessary to treat the cells a second time with Mynox®. In that case it is important to provide enough time for recovery (two days/check with the microscope) to the cells after the first treatment.

When can I be certain that Mycoplasma is permanently eliminated?

In case that a few mycoplasma particles survive the treatment with Mynox® they will grow to detectable titers after four passages. You can detect mycoplasma at an early stage with the highly sensitive Venor®GeM Mycoplasma Detection Kit to exclude persisting contamination.

Do I have to remove standard antibiotics for the treatment with Mynox®?

No. Standard antibiotics like penicillin/streptomycin could be carried along during the treatment with Mynox®. As a basic principle, we would not recommend the use of antibiotcs. Pen/Strep mainly effects germs of the mouth and faucal cavity and was introduced in times, when laboratory staff used their mouth for pipetting. This situation changed with the introduction of pipetting-aids and security workbenches. Antibiotics can effect the cellular metabolism and thus the results of experiments. (compare with: Kuhlmann, Cytotechnology 19:95-105,1996 „The prophylactic use of antibiotics in cell culture"). Bacterial contaminations can

interfere latently and unrecognized. With Onar®*EUB* Minerva Biolabs provides a sensitive PCR-Detection Kit for bacterial contamination (Cat.-No. 12-1025).

Mynox® was uneffective in eliminating mycoplasma from virus suspensions.

The cell suspension must be free of cellular debris before treatment. Before the supernatant can be effectively treated, cellular debris should be centrifuged (1.000 rpm, 5 min) to form a pellet. Such debris as cell wall fragments will competively bind Mynox®, thus decreasing the effective concentration of the elimination reagent.

Mynox® was harmful to cells during treatment.

Most cases in which Mynox® was believed to be detrimental to cells, the mixture as prescribed in the Instruction Manual was not properly followed, thus resulting in a cytotoxic Mynox® concentration. Cells should be observed frequently during treatment. If cytotoxic effects are clearly evident, the treatment should be immediately stopped by medium change. For cells known to be sensitive to Mynox®, the treatment time should be reduced by passage of cells after 30 minutes for adherent cell lines and 15 minutes for suspension cell lines.

Can the cellular concentration be increased for treatment with Mynox®?

The cell concentration may be increased by 10-fold, however an overall decrease in the elimination efficiency of Mynox® should subsequently be expected.

Can Mynox® eliminate intracellular contaminants?

Mynox® does not integrate into the cellular membrane. Therefore it cannot eliminate intracellular contamination. However, mycoplasma is an extracellular contaminate. Mycoplasma penetrans and Mycoplasma gallisepticum are the only species described intracellulary. Both M. penetrans and M. gallisepticum have not been reported as cell contaminants.

Is Mynox® effective against bacteria, fungi, or chlamydia?

No, Mynox® is only effective against mycoplasma. Mynox® is especially useful against mycoplasma contamination in chlamydia cultures, as standard antibiotic treatments are damaging to chlamydia in cell lines.

Can primary cells be treated with Mynox®?

Yes, primary cells can be treated with Mynox®. However, we recommend a 10-fold increase in the cell concentration. (Note that an overall decrease in the elimination efficiency of Mynox® should subsequently be expected).

Can trypsin be a possible source of contamination in cell cultures?

Trypsin is derived from swine sources and believed to be a source of Mycoplasma hyorhinis contamination. However, Mycoplasma hyorhinis is lysed at room temperatures by trypsin within minutes, thus it presents no source of contamination.

Can mycoplasma contamination be observed with the naked eye?

No, mycoplasma can only be observed through electron microscopy. For highly sensitive detection of mycoplasma contamination, we recommend the Venor®*GeM* Mycoplasma Detection Kit.

Mycoplasma-Off®

What are recommended methods of eliminating mycoplasma contamination on surfaces or other laboratory apparatus?

Mycoplasma-Off® Surface Disinfection Spray is ideal for cleansing and disinfection of all laboratory surfaces and apparatus including clean benches, incubators, work benches, cell storage boxes and liquid nitrogen containers. Mycoplasma-Off® is also effective against a broad range of other pathogenic contaminants.

What is the principle of Mycoplasma-Off®? How does it kill mycoplasma and how long will mycoplasma survive in general?

Mycoplasma-Off® contains membrane active components acting in combination with the alcohols included. It also includes aldehydes mainly for the inactivation of non-enveloped viruses and spores. Mycoplasma-Off® is also active against bacteria and other microorganisms. It is not quite understood how long mycoplasma can survive outside a culture or their natural habitat. By our knowledge no study is available. It is only known that cross contamination is very prominent for cell cultures. That can only be the case with quite stable mycoplasma.