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ProductInformation

Mynox® Mycoplasma Elimination Reagent

Product Code M 8190
Storage Temperature 2-8 °C

Product Description

For both biological and economical reasons, it is important to eliminate mycoplasma from cell cultures being used for basic research, diagnostics, and biotechnological production. The most commonly used method for elimination, inactivation, or suppression of mycoplasma in cell cultures is treatment with antibiotics. In general, antibiotic therapies do not result in long-lasting, successful elimination. Also, the cytotoxic properties of antibiotics can cause undesirable side effects on eukaryotic cells and can facilitate the development of resistant mycoplasma strains.

Mynox® is the first biological reagent that actually eliminates mycoplasma by killing them. It has been shown to be effective with only one treatment. Mynox® activity is based on its biophysical properties, making the development of resistant strains highly unlikely and is easily removed after the treatment.

In comparison to mammalian cells, mycoplasma lack a cell wall but are encircled by a cytoplasmic membrane. Mynox® is a biological agent that integrates into the mycoplasma membrane and compromises its integrity. The result is an osmotic influx that leads to the complete disintegration of the mycoplasma membrane. With mycoplasma eradication, mammalian cells immediately return to their native morphology and normal proliferation rates. To date, Mynox® has not been shown to cause any changes in normal cell characteristics.

Intended Use

Mynox® is intended for research use only. Mynox® is used for the elimination of *Mycoplasma* and *Acholeplasma* in cell and virus cultures, and other biologicals.

Components

Kit components are an Instruction Manual and Mynox® Reagent which is a sterile, ready-to-use solution in phosphate-buffered saline (PBS), pH 7.4. The reagent is dispensed in aliquots of 220 µl per tube for single application use:

Product Code M 8190	2 treatments

Storage/Stability

Upon receipt, store at 2-8 °C. By following these recommendations, Mynox® is stable until the expiration date stated on the label.

Procedure

Adherent Cell Lines: Preparation, Treatment, and Mynox® Removal

Prepare cells and the elimination mix in a sterile 6-cm petri dish. Add 2.8 ml of standard cell culture medium with 5 % FBS (v/v) and 200 µl Mynox® Reagent to the dish. Transfer 2 ml of 1 x 10^4 to 1 x 10^5 of freshly trypsinized cells in cell culture medium with 5% FBS (v/v) into the mix. The total volume of the treatment mixture is 5 ml.

Important:

1. Ensure the treatment of single cells by checking them under a microscope. If necessary, increase the duration of trypsin treatment or detach the cells from each other by pipetting up and down.
2. Ensure that Mynox® is already present in the culture medium before adding cells. Add cells directly to the elimination mix to avoid evaporation.

After 2 hours of incubation under normal growth conditions, remove the elimination mix by discarding the supernatant. Overlay the cells with standard cell culture medium. For the most effective method, maintain the cells in the elimination mixture for one entire passage (approximately 3-8 days) under normal growth conditions. Then remove the medium containing Mynox® and subculture the cells in standard medium as normal.

Important:

During treatment the cell culture should be checked frequently for cytotoxic effects and, if clearly noticable, the reaction stopped immediately by medium change or 1:5 dilution of the mixture with medium.

Suspension Cell Lines: Preparation, Treatment, and Mynox Removal

Prepare cells and the reaction mix in a sterile centrifuge tube. Add 1.6-ml mix of 0.125% trypsin and 5 mM

EDTA in PBS and 200 µl of Mynox® Reagent to the tube.
Transfer 1.6-ml standard cell culture medium with 10 % FBS (v/v) and 1 x 10^4 to 1 x 10^5 cells from a suspension cell line into the mixture. The total volume of the treatment mixture is 3.4 ml.

Important:

1. Ensure the treatment of single cells by checking under a microscope.
2. Ensure that Mynox® is already present in the culture medium before adding cells. Add cells directly to the elimination mix to avoid evaporation.
3. Ensure that the elimination mixture wets the complete inner surface of the centrifuge tube.
4. Trypsin is needed to avoid cell aggregates. If cell separation can be achieved by other techniques replace the trypsin volume with cell culture medium before adding the cells to the elimination mix. In any case the total volume of the elimination mix has to be 3.4 ml, if necessary add PBS.

For treatment and Mynox® removal, shake the mixture gently at room temperature for 30 min. Pellet the cells gently by centrifugation (600 x g, 5 min) and discard the supernatant. Resuspend the cells in Mynox®-free standard cell culture medium. For a more effective method, subcultivation in the presence of Mynox® for 1 passage is possible. To do this, resuspend the cells in 5 ml cell culture medium containing 5 % FBS (v/v) and 150 µl Mynox®. Incubate the cells in this medium for 3 days in a culture flask under normal growth conditions followed by separating the cells from the elimination mixture by centrifugation, then subculture the cells in Mynox® reagent-free growth medium.

Important:

During treatment the cell culture should be checked frequently for cytotoxic effects and, if clearly noticable, the reaction stopped immediately by medium change or 1:5 dilution of the mixture with medium.

Treatment of Non-enveloped Viruses

Frozen or fresh aliquots of cell and cell debris-free virus suspensions can be treated. The virus titer does not influence the success of the treatment. Prepare reaction mix by adding 1-ml cell culture medium without FBS and 100 µl Mynox® reagent to a sterile 1.5-ml reaction tube with safety-lock tops. Transfer 125-µl virus stock, containing up to 8 % FBS into the mixture**.** The total volume of the treatment mixture is 1.225 ml.

Important:

Ensure that the elimination mixture wets the complete inner surface of the reaction tube.

For treatment and Mynox® removal, incubate the elimination mixture at room temperature by gentle shaking for 2 hours. The reaction is stopped by diluting Mynox® 1:10 in culture medium. This step can be accomplished by using the elimination mixture to infect a subconfluent, host cell culture for simultaneous propagation of the mycoplasma free virus culture. Final volume should be 10X that of the elimination mixture.

Important:

Test the host cell line for mycoplasma contamination prior to infection.

Treatment of Enveloped Viruses

The composition of the outer lipid membrane of enveloped viruses is comparable to the mycoplasma membrane, the target of Mynox®. These viruses are also vulnerable to Mynox® inactivation depending on the treatment time and concentration used. To achieve mycoplasma-free virus suspensions with an acceptable level for subcultivation, the initial virus titer should be higher than 10^6 $TCID_{50}$.

Frozen or fresh aliquots of cell and cell debris-free virus suspensions can be treated. In a sterile 15-ml screw cap reaction tube mix 4.4 ml cell culture medium containing no FBS and 100 µl Mynox®. Transfer 0.5-ml virus stock, containing up to 8 % FBS into mixture. The total volume of the treatment mixture is 5 ml.

Important:

Ensure that the elimination mixture wets the complete inner surface of the reaction tube.

For treatment and Mynox® removal, incubate the reaction mixture at room temperature by gentle shaking for 2 h. The reaction is stopped by diluting Mynox® 1:10 in culture medium. This step can be accomplished by using the elimination mixture to infect a subconfluent culture of the host cell line for simultaneous propagation of the mycoplasma free virus culture. Final volume should be 10X that of the elimination mixture.

Important:

1. Test the host cell line for mycoplasma contamination prior to infection.
2. These mycoplasma elimination procedures can be repeated with the viruses directly harvested from the host cell cultures to ensure that all mycoplasma have been removed.

Treatment of other Biologicals

For samples with low protein and lipid concentrations, we recommend applying Mynox® in a 1:50 dilution. But out of the diversity of possible biologicals where Mynox® is applicable, we can only give general recommendations for Mynox® applications. The most

appropriate protocol must be optimized for individual cases.

Testing for Mycoplasma
For highly sensitive detection of mycoplasma contamination, we recommend VenorGeM® Mycoplasma Detection Kit (Product Code MP0025) that uses PCR technology. Mynox®-treated cell cultures and virus stocks should be subcultivated for three additional passages without antibiotics and then assayed for mycoplasma re-emergence to validate culture purity.

Sample Material
Importance of Serum Concentration
The mycoplasmacidal activity of Mynox® is affected by the concentration of lipids and proteins in the reaction mixture, e.g., components in fetal bovine serum supplement. These ingredients competitively bind Mynox® and prevent its binding to the mycoplasma membrane. Therefore, the protocol for mycoplasma elimination in cell cultures was designed for a specific standard cell culture medium, e.g. Dulbecco´s modified Eagle´s medium (D-MEM; Product Code D 5796) or RPMI-1640 (Product Code R 8758). For the treatment of cell lines, a protocol was designed that requires a supplement of 5 % FBS (v/v). For virus stocks, it is highly recommended that the medium be almost free of supplemental serum during treatment.

Limits of Mynox®
Mynox® will not eliminate the cell penetrating *Mycoplasma penetrans*. Also, due to the mitigating effect of serum, it is impossible to design a specific protocol that is applicable for the treatment of biologicals with high protein and lipid concentrations. Since Mynox® works by biophysical means through association with the mycoplasma membrane, the reagent needs direct contact with the mycoplasma particle in order to be effective. Treatment of cell clusters should be avoided. Mycoplasma are protected in intercellular spaces as well as in pockets and clefts of the cell membrane, which can prevent contact with the drug. We suggest using trypsin to detach the cells from each other and to smooth cell surfaces.

Cytotoxity of Mynox®
Similar to all other products available for mycoplasma inactivation, Mynox® also shows a cytotoxic effect on adherent and nonadherent cell lines. Our protocols were tested on numerous cell lines and found to have a cytotoxicity between 10-80%, with enough viable cells recovered for further subcultivation. Generally, higher proliferation rates as a result of parasite removal will compensate for lost cell material.

Precautions and Disclaimer
MSDS is available upon request at www.sigma-aldrich.com. Mynox is a registered trademark of Minerva Biolabs GmbH, Berlin, Germany.
The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffman-LaRoche. Use of the PCR process requires a license.