Mynox® Gold

Elimination of Mycoplasma in Cell Culture

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL
Symbols

Lot No.

Order No.

Expiry date

Storage temperature

Number of reactions

Manufacturer
INDICATION

Contamination of cell cultures by mycoplasma occurs frequently. For both safety 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 therapy alone does not result in long-lasting, successful elimination. Also, the cytotoxic properties of antibiotics can cause undesirable side effects in eukaryotic cells, and may facilitate the development of resistant mycoplasma strains.

PRINCIPLE OF THE METHOD

Mynox® Gold represents the further development of the classical Mynox® reagent. Mynox® Gold is a combination of an antibiotic and a biological reagent. In comparison to mammalian cells, mycoplasma lack a cell wall but are encircled by a cytoplasmic membrane. The biological reagent of Mynox® Gold integrates into the mycoplasma membrane and compromises its integrity. By the combination with an antibiotic, the effective dose of both, the reagent and the antibiotic, can be reduced to a minimum for lowest cytotoxicity, while still causing a highly reliable and definite elimination of mycoplasma. These biophysical properties make the development of resistant strains highly unlikely.

One application of Mynox® Gold comprises of 4 vials, a Starter Treatment and three Main Treatments. The Starter Treatment kills most of the mycoplasma particles without harming the cells. The Main Treatment kills all remaining particles leading to a permanent mycoplasma elimination in the treated cell culture.
REAGENTS AND COMPONENTS

Each Mynox® Gold kit contains 2, 5, or 10 packs with Mynox® Gold reagents (1 pack for 1 treatment). Each pack contains 1 vial Starter Treatment and 3 vials Main Treatment. Each component is a sterile, ready-to-use solution, aliquoted per vial for single applications of 520 µl/vial.

Mynox® Gold is shipped at room temperature, and stable until the expiry date when stored at +2 to +8 °C in a dark environment. The expiry date is marked on the package label.

<table>
<thead>
<tr>
<th>Component</th>
<th>2 packs Cat. No. 10-0201</th>
<th>5 packs Cat. No. 10-0501</th>
<th>10 packs Cat. No. 10-1001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mynox® Gold Starter Treatment reagent</td>
<td>2 vials (1 vial/pack, 520 µl/vial)</td>
<td>5 vials (1 vial/pack, 520 µl/vial)</td>
<td>10 vials (1 vial/pack, 520 µl/vial)</td>
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<tr>
<td>Mynox® Gold Main Treatment reagent</td>
<td>6 vials (3 vials/pack, 520 µl/vial)</td>
<td>15 vials (3 vials/pack, 520 µl/vial)</td>
<td>30 vials (3 vials/pack, 520 µl/vial)</td>
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USER-SUPPLIED CONSUMABLES AND EQUIPMENT

- Standard cell culture equipment (incubator, water bath, disposable serological pipets)
- Sterile 25 cm² cell culture flask or 10 cm petri dish for cell culture
- Sterile 15 ml conical tubes (Falcon type)
- Pipettes with corresponding filter-tips (1000 µl)
- Cell culture medium
- Fetal calf serum (FCS)
- Trypsin
- Mycoplasma detection system to verify the elimination success, e.g. Minerva Biolabs Venor®GeM mycoplasma PCR detection kits (see "Related Products" for ordering information).
The product is used for the elimination of all kinds of Mollicutes and related organisms (e.g. Mycoplasma, Acholeplasma, Spiroplasma, and Entomoplasma) in all kinds of cell and virus cultures. For best results the following recommendations should be considered:

1. The cell number used for the treatment should not exceed $10^5$ cells in order to keep the mycoplasma load low.

2. The mycoplasmacidal activity of Mynox® Gold is affected by the concentration of lipids and proteins in the reaction mixture, e.g. components in fetal calf serum (FCS) supplement. These ingredients competitively bind Mynox® Gold and prevent its binding to the mycoplasma membrane. Therefore, a protocol was designed, in which a supplement of 5 % v/v fetal calf serum in cell culture medium is required. 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.

3. The type of cell culture medium does not affect the efficiency of the treatment. Antibiotics, especially if required for selection, can be maintained in the treatment mixture. In rare cases, cytotoxicity might be increased by unpredictable interactions of the reagents.

4. Viruses should be treated in combination with their host cells. Protocols for the direct treatment of non-enveloped and enveloped viruses are available upon request.

5. Mynox® Gold does not penetrate the cellular membrane. Therefore, it cannot eliminate intracellular contaminations. However, mycoplasma are known to be extracellular contaminants. Although mycoplasma cell-invasion events were recently reported, those have been shown in field isolates only, and after 2 to 3 passages, mycoplasma were unable to invade the cell membrane. In addition, unlike Legionella and Chlamydia, the mycoplasma genome does not contain any invasion-related genes. In fact, several proteins known to mediate adhesion to the surface of the host cell are prominent within the mycoplasma family. Therefore, all mycoplasma contaminations in permanent cell lines are due to mycoplasma adhesion to cell surface or to mycoplasma presence in cell culture supernatant. Either way, such contaminations could easily be eliminated by using Mynox® Gold reagent.

6. Since Mynox® Gold 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 smoothen cell surfaces.
RECOMMENDATIONS

Regular monitoring of mycoplasma contaminations in cell culture is essential for contamination control and to ensure the maintenance of a mycoplasma-free cell culture. It is also recommended to check other user supplied biologicals, like fetal calf serum (FCS), for contaminations on a regular basis. We therefore recommend our PCR-based Venor®GeM mycoplasma detection kits for a highly sensitive detection of mycoplasma contamination (see Related Products for ordering information).

Mynox® Gold is intended for research use only.

PRECAUTIONS

Mynox® Gold should be used by trained laboratory staff only. All samples should be handled with all due care and attention. Always wear a suitable lab coat and disposable gloves. This kit does not contain hazardous substances. Waste is disposable according to local regulations.

ADDITIONAL NOTES

⇒ These instructions must be understood to successfully use Mynox® Gold. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit.

⇒ Mynox® Gold reagents are light-sensitive and should be stored in a dark environment. They should not be used beyond their shelf life.

⇒ Follow the exact protocol. Deviations may affect the results.
PROCEDURE – OVERVIEW

1. Prepare Treatment Mix
   - Starter Treatment
     Mynox® Gold 500 µl
   - 4.5 ml medium
     (5 % v/v FCS)
   - vortex

2. Load Culture Plate or Flask
   - 25 cm²
   - or
   - 10 cm
   - 5 ml
   - Mynox® Gold / medium mix

3. Add Cells
   - 10⁴-10⁵ cells
     in 5 ml medium
     (5 % v/v FCS !!!)

   - cell passage
     9.5 ml medium
     + Mynox® Gold 500 µl
   - Main Treatment

   - culture cells as usual
     to 80 - 90 % confluency
   - passage cells
     cultivate Mynox® Gold free

   - test with Venor®GeM Mycoplasma Detection Kit

   - repeat main treatment 2×

Storage 2 - 8 °C, dark environment

! Ensure treatment of single cells (check under microscope).
!! Attention: insert pipet tip directly into treatment mix. Do not touch the inner wall of the flask/petri dish with pipet tip.
!!! Control FCS concentration.

This procedure overview is not a substitute for the detailed manual.
PROCEDURE - STEP BY STEP

These protocols have been designed for typical cell lines requiring standard media. The protocol can be used for adherent and suspension cell lines. Minerva Biolabs does not guarantee that these protocols will work in all laboratory situations. Modification of these protocols may be required per individual case.

1. Preparation of Starter Treatment Mix

- Pipet 4.5 ml standard cell culture medium with 5 % v/v FCS into a sterile 15 ml conical tube (Falcon type), and add 500 µl Mynox® Gold Starter Treatment (vial with orange cap).
- Vortex Mynox® Gold / medium mix shortly.
- Transfer Mynox® Gold / medium mix (5 ml) into a sterile 25 cm² cell culture flask or into a sterile 10 cm petri dish.

2. Addition of Cells

- Passage cells as usual and use trypsin to disassociate cell clusters into single cells.
- Attention: Check under microscope to ensure the treatment of single cells. If necessary, increase duration of trypsin treatment or detach the cells from each other by carefully pipetting up and down.
- Transfer 10⁴ – 10⁵ single cells in 5 ml cell culture medium with 5% (v/v) FCS into the Mynox® Gold / medium mix. The total volume of the treatment mixture is 10 ml and final FCS concentration is 5 % (v/v).
- Attention: Insert pipet tip directly into the treatment mix to avoid aerosols. Make sure you do not touch the inner wall of the flask or petri dish with pipet tip.
- Agitate the flask or petri dish by gently rocking back and forth and sideways to avoid uneven distribution of cells. Incubate cells as usual to proceed with Mynox® Gold Main Treatment.

3. Main Treatment

- Culture cells to 80 – 90 % confluency.
- Split the cells and passage at the usual rate. Add 500 µl of the Mynox® Gold Main Treatment reagent (vial with transparent cap) to 9.5 ml of passaged cells in fresh medium. Adjust the FCS concentration. Supplementation to 5 % (v/v) FCS is not required any longer.
- Repeat Mynox® Gold Main Treatment two more times. After the third treatment (and a total of 4 passages starting with the starter treatment), the procedure is finished and the culture is free of mycoplasma.
4. Testing for Mycoplasma

Mynox® Gold-treated cell cultures and virus stocks should be subcultivated for four additional passages without mycoplasma-active antibiotics and then assayed for mycoplasma re-emergence to validate culture purity. For highly sensitive detection of mycoplasma contamination, we recommend our PCR-based Venor®GeM mycoplasma detection kits (see Related Products for ordering information).

1. PCR detection methods should not be used instantly after treatment. Mynox® Gold lyses mycoplasma particles and the mycoplasma DNA is subsequently released into the culture medium. This DNA would be detected by PCR giving false-positive results. Medium replacement and extracellular DNases will reduce the level of free mycoplasma DNA within 1 to 2 passages.

2. Repeat PCR mycoplasma detection assay at regular intervals to ensure the maintenance of a mycoplasma-free cell culture.

APPENDIX

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Trademarks

Mynox, Venor, Onar, ZellShield are registered trademarks and Mycoplasma Off, PCR Clean and WaterShield are trademarks of Minerva Biolabs GmbH, Germany.
Related Products

**MB Taq DNA Polymerase**
- 53-0050/-0100/-0200/-0250 MB Taq DNA Polymerase (5 U/µl) 50/100/200/250 units
- 53-1050/-1100/-1200/-1250 MB Taq DNA Polymerase (1 U/µl) 50/100/200/250 units

**Detection Kits for conventional PCR**
- 11-1025/1050/1100/1250 Venor®GeM Classic 25/50/100/250 tests
- 11-7024/7048/7096/7240 Venor®GeM Advance 24/48/96/240 tests
- 11-8025/8050/8100/8250 Venor®GeM OneStep 25/50/100/250 tests
- 12-1025/1050/1100/1250 Onar®Bacteria 25/50/100/250 tests

**Mycoplasma Detection Kit for qPCR**
- 11-9025/9100/9250 Venor®GeM qEP 25/100/250 tests

**Mycoplasma Elimination**
- 10-0200/0500/1000 Mynox® Mycoplasma Elimination Reagent 2/5/10 treatments
- 10-0201/0501/1001 Mynox® Gold Mycoplasma Elimination Reagent 2/5/10 treatments

**PCR Quantification Standards, lyophilized, 1x10^8 genomes / vial**
- 52-0116 Acholeplasma laidlawii
- 52-0115 Mycoplasma gallisepticum
- 52-0129 Mycoplasma arginini
- 52-0117 Mycoplasma fermentans
- 52-0130 Mycoplasma hyorhinis
- 52-0112 Mycoplasma orale
- 52-0119 Mycoplasma pneumoniae
- 52-0124 Mycoplasma synoviae
- 52-0164 Spiroplasma citri

See Minerva homepage for further available species

**10CFU™ Sensitivity Standards, 3 vials with 10 CFU each, 2 vials negative control**
- 102-1003 Mycoplasma arginini
- 102-2003 Mycoplasma orale
- 102-3003 Mycoplasma gallisepticum
- 102-4003 Mycoplasma pneumoniae
- 102-5003 Mycoplasma synoviae
- 102-6003 Mycoplasma fermentans
- 102-7003 Mycoplasma hyorhinis
- 102-8003 Acholeplasma laidlawii
- 102-9003 Spiroplasma citri
- 102-0002 Mycoplasma Set, all EP 2.6.7 listed species 2 vials per species, 10 CFU each

**PCR Clean™ (formerly DNA Remover™)**
- 15-2025 DNA Decontamination Reagent, spray bottle 250 ml
- 15-2200 DNA Decontamination Reagent, refill bottles 4 x 500 ml

**Mycoplasma Off™**
- 15-1000 Surface Disinfectant Spray, spray bottle 1000 ml
- 15-5000 Surface Disinfectant Spray, refill bottles 5 x 1000 ml
- 15-1001 Surface disinfectant wipes in dispenser box 120 wipes
- 15-5001 Surface disinfectant wipes, refill pack 5 x 120 wipes
- 15-1030 Wipes, separately wrapped 30 sachets

**ZellShield®**
- 13-0050/-0150 Contamination Prevention Reagent 100× concentrate 1000 ml/ 5 x 1000 ml

**WaterShield™**
- 15-3015/3020/3050 Water Disinfection Additive for incubators and water baths, 200x concentrate 30 x 5 ml/3 x 50 ml/500 ml