Mynox® Gold

Elimination of Mycoplasma in Cell Cultures

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. 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 used in 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, however, antibiotic treatment alone does not result in long-lasting, successful elimination of contaminating mycoplasma. 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 a further development of the classic Mynox® reagent, combining an antibiotic and a biological reagent with a biophysical mode of action. In comparison to other bacterial cells, mycoplasma lack a cell wall but are encircled by a plasma membrane. The biological reagent contained in Mynox® Gold integrates into the mycoplasma membrane and compromises its integrity. Due to this combined formulation, the effective dose of both, the biological reagent and the antibiotic, can be reduced to a minimum for lowest cytotoxicity, while still reliably and definitely eliminating mycoplasma. The biophysical mode of action of the reagent minimizes the risk of the development of resistant strains to negligible levels.

One application of Mynox® Gold consists of 4 vials: one Starter Treatment and three Main Treatments. The starter Treatment destroys most of the mycoplasma particles whereas the Main Treatment irreversibly damages all remaining particles leading to a permanent elimination of mycoplasma from the treated cell culture. Both treatments are harmless to the cells in culture.

REAGENTS

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.

Component	2 packs Cat. No. 10-0201	5 packs Cat. No. 10-0501	10 packs Cat. No. 10-1001
Mynox® Gold Starter Treatment reagent	2 vials (1 vial/pack, 520 µl/vial)	5 vials (1 vial/pack, 520 µl/vial)	10 vials (1 vial/pack, 520 µl/vial)
Mynox® Gold Main Treatment reagent	6 vials (3 vials/pack, 520 µl/vial)	15 vials (3 vials/pack, 520 µl/vial)	30 vials (3 vials/pack, 520 µl/ vial)

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

- Standard cell culture equipment (incubator, water bath, disposable serological pipets)
- Sterile 25 cm^2 cell culture flask or 6 cm petri dish for cell culture
- Sterile 15 ml conical tubes
- Pipettes with corresponding filter-tips (1000 µl)
- Cell culture medium
- Fetal calf serum (FCS)
- Trypsin
- Cell counting equipment and conventional microscope
- Mycoplasma detection assay to verify the elimination success, e.g. Minerva Biolabs Venor®GeM mycoplasma PCR detection kits (see "Related Products" for ordering information).

SPECIMEN

The product is used for the elimination of all species belonging to the *Mollicutes* class and related organisms (e.g. *Mycoplasma, Acholeplasma, Spiroplasma, and Entomoplasma*) in any type 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 total cells (for each 25 cm^2 cell culture flask or 6 cm petri dish). This guarantees a low mycoplasma load.

2. The mycoplasmacidal activity of Mynox® Gold is affected by the concentration of lipids and proteins in the reaction mixture, e.g. components of culture media supplements like fetal calf serum (FCS). These ingredients competitively bind the biological reagent contained in Mynox® Gold and prevent its binding to the mycoplasma membrane. Therefore, we recommend using our extensively tested protocol with 5 % v/v FCS in cell culture medium. Due to the inhibitory effect of serum proteic and lipidic components, a specific protocol for the treatment of biologicals with high protein and lipid concentrations is not available.

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, the reagent remains inactive against intracellular contaminations. However, mycoplasma are known to be extracellular contaminants. Although mycoplasma invasive-like behavior was recently reported, such events have been shown in isolated fields, only and ended after 2-3 cell culture passages, when mycoplasma were no longer shown 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 prominently expressed within members of the mycoplasma family. Altogether, this indicates a rather adhesive-like interaction of mycoplasma with the membrane of host cells and suggests that, in contaminated cell lines, these microorganisms are located perimembranously and/or extracellularly (cell culture supernatant). Both localizations of the contaminants are compatible with an effective treatment with Mynox® Gold reagent.

6. Since the mode of action of Mynox® Gold is based on its physical complexation with the mycoplasma membrane, an effective treatment requires direct contact of the reagent with the mycoplasma particles. Treatment of cell clusters should be avoided. Mycoplasma can accumulate in intercellular spaces as well as in pockets and clefts of the cell membrane, thereby escaping contact with the drug. We suggest dissociating the cell clusters with trypsin and mechanical disgregation in order to reduce such intercellular gaps and smoothen cell surfaces.

RECOMMENDATIONS

Regular monitoring of mycoplasma contaminations in cell cultures and other user-supplied biologicals, like FCS or trypsin, is essential for contamination control and to ensure the maintenance of a mycoplasma-free cell culture. We recommend our PCR-based Venor®GeM mycoplasma detection kits for 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

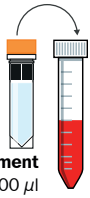
⇒ These instructions must be understood to successfully use Mynox® Gold. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit.

⇒ 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

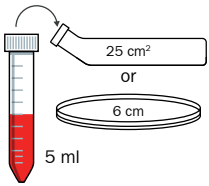


Starter Treatment
Mynox® Gold 500 µl

4.5 ml medium
(5 % v/v FCS)

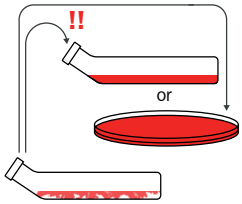
vortex

2. Load Culture Plate or Flask



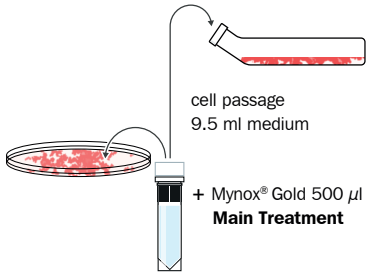
Mynox® Gold / medium mix

3. Add Cells



10^4-10^5 cells !
in 5 ml medium
(5 % v/v FCS !!!)

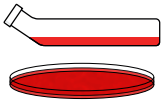
culture cells as usual
to 80 - 90 % confluency



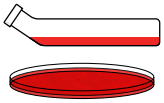
cell passage
9.5 ml medium

+ Mynox® Gold 500 µl
Main Treatment

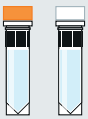
repeat main treatment 2×



passage cells
cultivate Mynox® Gold free



test with Venor®GeM Mycoplasma Detection Kit



Storage +2 - +8 °C, dark environment

! Ensure treatment of single cells (check under microsope).
!! 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.

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PROCEDURE - STEP BY STEP

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

1. Preparation of Starter Treatment Mix

1.	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).
2.	Vortex Mynox® Gold / medium mix shortly.
3.	Transfer Mynox® Gold / medium mix (5 ml) into a sterile 25 cm^2 cell culture flask or into a sterile 6 cm petri dish.

2. Addition of Cells

	Passage cells as usual and use trypsin to dissociate cell clusters and obtain single cells.
1.	**<u>Attention</u>: Check under microscope to ensure the treatment of single cells. If necessary, increase duration of trypsin treatment or mechanically disgregate cell clusters by carefully pipetting up and down.**
2.	Transfer 10^4 – 10^5 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 the final FCS concentration is 5 % (v/v). **<u>Attention</u>: Insert pipet tip directly into the treatment mix to avoid aerosols. Make sure not to touch the inner wall of the flask or petri dish with pipet tip.**
3.	Gently rock the flask or petri dish back and forth and sideways to avoid uneven distribution of cells. Incubate cells as usual and proceed to **Mynox® Gold Main Treatment.**

3. Main Treatment

1.	Culture cells to 80 – 90 % confluency.
2.	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.
3.	Repeat **Mynox® Gold Main Treatment** two more times. After the third treatment (and a total of 4 passages starting with the Starter Treatment), the mycoplasma elimination procedure is completed.

4. Testing for Mycoplasma

1. Mynox® Gold-treated cell cultures and virus stocks need to be subcultivated for four additional passages without antibiotics against mycoplasma before they can be assayed for mycoplasma re-emergence. For highly sensitive detection of mycoplasma contamination, we recommend our PCR-based Venor®GeM mycoplasma detection kits (see Related Products for ordering information).

   Please note: PCR-based detection methods should not be used directly after treatment. Mynox® Gold lyses mycoplasma particles, which subsequently release mycoplasma DNA into the culture medium. The free DNA can then be detected by PCR, leading to false-positive results. Medium replacement and extracellular DNases guarantee washout and progressive reduction 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. Falcon is a registered trademark of Corning Inc.

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
Contamination Control Kits for conventional PCR		
11-1025/-1050/-1100/-1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 reactions
11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 reactions
11-8025/-8050/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 reactions
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 reactions
Contamination Control Kits for qPCR		
11-9025/-9100/-9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 reactions
11-91025/-91100/-91250	Venor®GeM qOneStep Mycoplasma Detection Kit	25/100/250 reactions
Sample Preparation		
56-1010/-1050/-1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
Mycoplasma Elimination		
10-0200/-0500/-1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
ZellShield®		
13-0050/-0150	Contamination Prevention Reagent 100x concentrate	50 ml/ 3 x 50 ml
WaterShield™		
15-3015/-3020/-3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	15 × 10 ml/3 × 50 ml/500 ml
Mycoplasma Off™		
15-1000/-5000	Surface Disinfectant, Spray bottle/refill bottles	1 l/5 l
15-1001	Surface Disinfectant, Wipes in a dispenser box	120 wipes
15-5001	Surface Disinfectant, Wipes in refill bags	5 × 120 wipes
PCR Clean™		
15-2025/-2200/-2500	DNA Decontamination Reagent, Spray bottle/refill bottles	250 ml/4 × 500 ml/5 l
15-2001	DNA Decontamination Reagent, Wipes in a dispenser box	120 wipes
15-2002	DNA Decontamination Reagent, Wipes in refill bags	5 × 120 wipes

PCR Quantification Standards, 1x10^8 genomes / vial

52-0112	*Mycoplasma orale*
52-0115	*Mycoplasma gallisepticum*
52-0116	*Acholeplasma laidlawii*
52-0117	*Mycoplasma fermentans*
52-0119	*Mycoplasma pneumoniae*
52-0103	*Mycoplasma salivarium*
52-0124	*Mycoplasma synoviae*
52-0129	*Mycoplasma arginini*
52-0130	*Mycoplasma hyorhinis*
52-0164	*Spiroplasma citri*

See MB 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*	
102-1103	*Mycoplasma salivarium*	
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

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