Mynox®

Mycoplasma Elimination Reagent

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

Contaminations of cell cultures by mycoplasma occur frequently. For scientific, safety, and economical reasons, it is essential to eliminate mycoplasma from contaminated cell cultures 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, however, antibiotic treatment does not result in long-lasting, successful elimination of contaminating mycoplasma. Also, the cytotoxic properties of antibiotics can cause undesirable side effects on eukaryotic cells and may facilitate the development of resistant mycoplasma strains.

Mynox® is the first biological reagent, which eliminates mycoplasma contaminations by inducing the microorganism death. It has been shown to be effective already with one treatment, only. Mynox® activity is based on its biophysical properties, which make the development of resistant strains highly unlikely.

PRINCIPLE OF THE METHOD

Mycoplasma lack a cell wall around their cytoplasmic membrane. Mynox® is a biological reagent that integrates into the mycoplasma membrane, compromising its integrity. The result is an osmotic influx leading to the complete disintegration of the mycoplasma membrane. Being mycoplasmas eradicated, cells can immediately resume their native morphology and normal proliferation rates. To date, Mynox® has not been shown to cause any changes in normal cell characteristics. Mynox® is used for the elimination of *Mycoplasma* and *Acholeplasma* in cell and virus cultures, and other biologicals. After treatment, Mynox® is easily removed by medium change.

REAGENTS

Mynox® reagent is a sterile, ready-to-use solution in phosphate-buffered saline (PBS), pH 7.4, aliquoted per vial for single applications.

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

Component	Cat. No. 10-0200	Cat. No. 10-0500	Cat. No. 10-1000
Mynox® reagent	2 vials (220 µl/vial)	5 vials (220 µl/vial)	10 vials (220 µl/vial)

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

- Standard cell culture equipment (incubator, water bath, pipettor, optionally vortex)
- Cell culture consumables according to application (e.g. sterile petri dishes, sterile cell culture flasks, 15 ml conical tubes etc.)
- Pipettes with corresponding filter-tips (1000 µl) and disposable serological pipettes
- Cell culture medium
- Fetal calf serum (FCS)
- Phosphate-buffered saline (PBS)
- 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).

SPECIMEN

1. 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 calf serum (FCS) supplement. These ingredients competitively bind Mynox® and prevent its binding to the mycoplasma membrane. Therefore, the protocol for mycoplasma elimination in cell cultures was established for specific standard cell culture media, e.g. Dulbecco Modified Eagle Medium (DMEM) or RPMI1640 and with 5 % v/v FCS. For virus stocks, it is highly recommended that the medium is almost free of supplemental serum during treatment. Due to the inhibiting effect of serum on Mynox® activity, a specific protocol applicable to the treatment of biologicals with high protein and lipid concentrations cannot be established. For the elimination of mycoplasma from serum-free cell cultures, we recommend testing Mynox® Gold. Please feel free to contact us for specific suggestions on the protocol to apply in these cases.

2. Limits of Mynox®

Mynox® does not cross the eukaryotic cell 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 allow an effective treatment with Mynox® reagent.

Since the mode of action of Mynox® 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 the cell surfaces.

3. Cytotoxicity of Mynox®

Similarly to all other products available for mycoplasma inactivation, Mynox® also shows a cytotoxic effect on adherent and non-adherent cell lines. Our protocols were tested on numerous cell lines and found to have a cytotoxicity between 10 and 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

Mynox® is intended for research use only. It 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

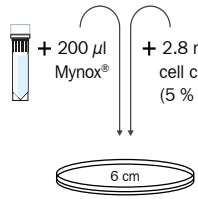
⇒ Regular monitoring of mycoplasma contaminations in cell culture is essential for contamination control and to ensure the maintenance of a mycoplasma-free cell culture lab. It is also recommended to check other reagents and biologicals, like fetal calf serum (FCS), for contaminations on a regular basis.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

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

⇒ Mynox® reagents should not be used beyond their shelf life.

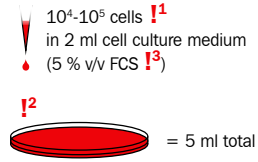
⇒ Follow the exact protocol. Deviations may affect the results.

1. Preparation of Mynox® elimination mix

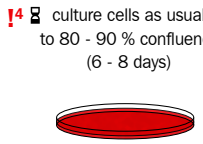


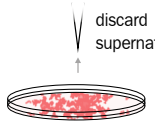
mix gently

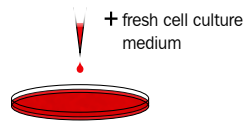
2. Adding cells

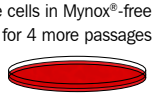


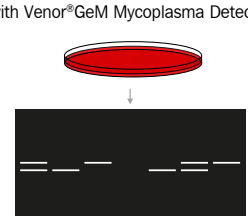
3. Mynox® treatment and removal











+ add
incubate

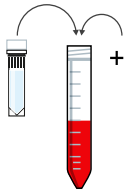
! Attention
1 Treat only single cells (check under microsope).
2 Add cells directly to elimination mix. Insert pipette tip directly into mix.
Do not touch the inner wall of the petri dish with pipette tip.
3 Control FCS concentration.
4 Check for cytotoxic effects during treatment and stop reaction in case of occurrence.

This procedure overview is not a substitute for the detailed manual.

MB_SI_Mynox_03_EN

1. Preparation of Mynox® elimination mix

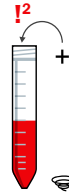
+ 200 µl Mynox®



+ 1.6 ml Trypsin (0.125 %, 5 mM EDTA)

mix

2. Adding cells

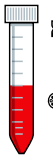


+ 10^4-10^5 cells !1 in 1.6 ml cell culture medium (10 % v/v FCS !3)

= 3.4 ml total

briefly !4

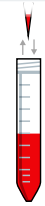
3. Mynox® treatment and removal !5



for 30 min at 37 °C

600 x g for 5 min

discard supernatant



+ 1-5 ml Mynox®-free fresh cell culture medium resuspend cells

transfer to flask culture cells in Mynox®-free media for 4 more cell passages

test with Venor®GeM Mycoplasma Detection Kit

4. For more effective results, subcultivate in the presence of Mynox® for 1 more passage:

resuspend cells in 5 ml cell culture medium

+ 150 µl Mynox®

+ 5 % (v/v) FCS

3 days as usual

culture cells in Mynox®-free media for 4 more cell passages

test with Venor®GeM Mycoplasma Detection Kit

centrifuge
vortex
+ add
incubate

! Attention

1 Treat only single cells (check under microsope).

2 Add cells directly to elimination mix. Insert pipette tip directly into mix. Do not touch the inner wall of the tube with pipette tip.

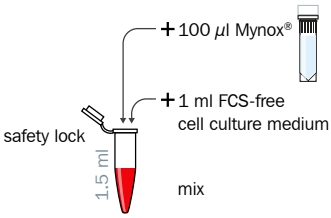
3 Control FCS concentration.

4 Vortex the elimination mix so that it moistens the complete inner surface of the tube.

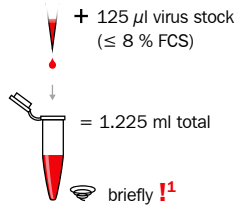
5 Check for cytotoxic effects during treatment and stop reaction in case of occurrence.

This procedure overview is not a substitute for the detailed manual.

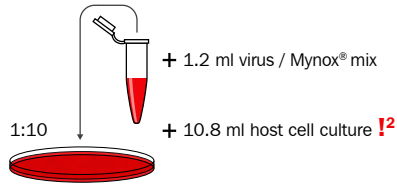
1. Preparation of Mynox® elimination mix



2. Adding virus stock

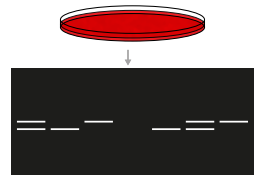


3. Mynox® treatment and removal



culture cells in Mynox®-free media for 4 more cell passages

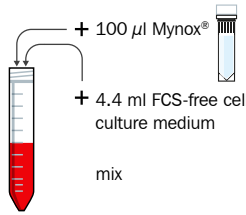
test with Venor®GeM Mycoplasma Detection Kit



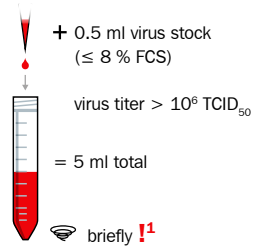
vortex
\+ add
incubate

! Attention
1 Vortex the elimination mix so that it moistens the complete inner surface of the tube.
2 Test the host cell line for mycoplasma contamination prior to infection.

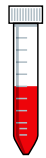
1. Preparation of Mynox® elimination mix

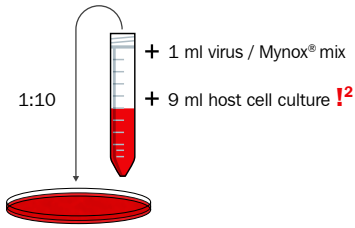


2. Adding virus stock



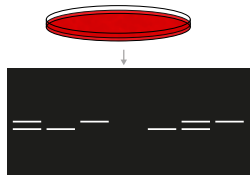
3. Mynox® treatment and removal





culture cells in Mynox®-free media for 4 more cell passages

test with Venor®GeM Mycoplasma Detection Kit



vortex
\+ add
incubate

! Attention
1 Vortex the elimination mix so that it moistens the complete inner surface of the tube.
2 Test the host cell line for mycoplasma contamination prior to infection.

This procedure overview is not a substitute for the detailed manual.

PROCEDURE - STEP BY STEP

These protocols have been established for typical cell lines, requiring standard media. Minerva Biolabs does not guarantee that these protocols can be successfully applied in all possible experimental conditions. Case-by-case optimization of these protocols may be required.

1. Treatment of Adherent Cell Lines

1.1 Preparation of cells and elimination mix

1.	Use a sterile 6 cm petri dish to prepare the elimination mix. **Do not use flasks.**
2.	Add 2.8 ml standard cell culture medium with 5 % (v/v) FCS.
3.	Add 200 µl Mynox® (1 vial).
4.	Transfer 2 ml of 10^4 – 10^5 freshly trypsinized cells in cell culture medium with 5 % (v/v) FCS into the mix. The total volume of the treatment mix including the cells is 5 ml. **<u>Attention</u>: Make sure to treat single cells only (check under microscope!). If necessary increase the duration of trypsin treatment or mechanically disgregate cell agglomerates by pipetting up and down.** **<u>Attention</u>: First add Mynox®, then the cells to the culture medium. Add the cells directly to the elimination mix (insert the pipette tip directly into the mix!).**

1.2 Mynox® treatment and removal

1.	Maintain the cells with the elimination mix for one entire passage (approximately 6 to 8 days) under normal growth conditions. Then, remove the Mynox®-containing medium and subculture the cells in standard medium as usual.
2.	**<u>Attention:</u> If cells do not reach confluency after 8 days, stop the treatment by discarding the Mynox®-containing medium and add fresh standard cell culture medium.** **<u>Attention</u>: During treatment, cells should be observed frequently to check for cytotoxic effects and, if clearly noticable, the treatment should be stopped immediately by medium change or 1:5 dilution of the mixture with medium.**

2. Treatment of Suspension Cell Lines

2.1 Preparation of cells and elimination mix

1.	Use a sterile centrifuge tube to prepare the elimination mix.
2.	Add 1.6 ml of 0.125 % trypsin and 5 mM EDTA.
3.	Add 200 µl Mynox® (1 vial).
4.	Transfer 1.6 ml standard cell culture medium with 10 % (v/v) FCS and 10^4 – 10^5 cells from a suspension cell culture into the elimination mix. Vortex briefly. The total volume of the treatment mix including the cells is 3.4 ml. **<u>Attention</u>: Make sure to treat single cells only (check under microscope!).** **<u>Attention</u>: First add Mynox®, then the cells to the culture medium. Add the cells directly to the elimination mix (insert the pipette tip directly into the mix!).** **<u>Attention</u>: Make sure the elimination mix moistens the complete inner surface of the centrifuge tube during the vortexing step.**

Trypsin is needed to disgregate cell clusters. If cell separation can be achieved by other techniques, replace the trypsin with the same volume of cell culture medium before adding the cells to the elimination mix. Please ensure that the total volume of the elimination mix is 3.4 ml. If necessary, add PBS.

2.2 Mynox® treatment and removal

1.	Incubate the mixture at 37 °C for 30 min.
2.	Pellet the cells by gentle centrifugation (600 × g for 5 min) and discard the supernatant.
3.	Resuspend the cells in Mynox®-free standard cell culture medium in a culture flask.

<u>For a more effective method</u>, a subcultivation in the presence of Mynox® for 1 passage is possible:

1.	Resuspend the cells in 5 ml cell culture medium containing 5 % (v/v) FCS and 150 µl Mynox®.
2.	Incubate the cells in this medium for 3 days in a culture flask under normal growth conditions.
3.	Subculture the cells in Mynox®-free growth medium.
4.	**<u>Attention</u>: During treatment, cells should be observed frequently to check for cytotoxic effects and, if clearly noticable, the reaction should be stopped immediately by medium change or 1:5 dilution of the mixture with medium.**

3. 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.

3.1 Preparation of the cells and the elimination mix

1.	Use a sterile 1.5 ml reaction tube **with safety-lock** to prepare the elimination mix.
2.	Add 1 ml cell culture medium without FCS.
3.	Add 100 µl Mynox®.
4.	Transfer 125 µl virus stock, containing up to 8 % (v/v) FCS into the mixture. Vortex briefly. The total volume of the treatment mix including the virus stock is 1.225 ml. **Attention: Make sure the elimination mix moistens the complete inner surface of the centrifuge tube during the vortexing step.**

3.2 Mynox® treatment and removal

1.	Incubate the elimination mixture at room temperature for 2 hours.
2.	The reaction is stopped by diluting the treatment mix 1:10 in culture medium. This can be accomplished by using the elimination mix to infect a subconfluent host cell culture for simultaneous propagation of the mycoplasma-free virus culture. Final volume should be 10 × that of the elimination mix. **Attention: Test the host cell line for mycoplasma contamination prior to infection.**

4. 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 obtain 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.

4.1 Preparation of cells and the elimination mix

1.	Use a sterile 15 ml screw-cap reaction tube to prepare the elimination mix.
2.	Add 4.4 ml cell culture medium without FCS.
3.	Add 100 µl Mynox®.
4.	Transfer 0.5 ml virus stock, containing up to 8 % (v/v) FCS into mixture. Vortex briefly. The total volume of the treatment mix including the virus stock is 5 ml. **Attention: Make sure the elimination mix moistens the complete inner surface of the centrifuge tube during the vortexing step.**

4.2 Treatment and Mynox® removal

1.	Incubate the elimination mixture at room temperature for 30 min.
2.	The reaction is stopped by diluting the treatment mix 1:10 in culture medium. This can be accomplished by using the elimination mix to infect a subconfluent culture of the host cell line for simultaneous propagation of the mycoplasma-free virus culture. Final volume should be 10 × that of the elimination mix. **Attention: Test the host cell line for mycoplasma contamination prior to infection.** **Attention: 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.**

5. Testing for Mycoplasma

Mynox®-treated cell cultures and virus stocks should be subcultivated for four additional passages without anti-mycoplasma antibiotics and then assayed for mycoplasma presence or 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).
Mynox® lyses mycoplasma particles subsequently leading to the release of mycoplasma DNA into the culture medium. This DNA can be detected by PCR resulting in false-positive results. PCR-based detection methods should not be used right after treatment. Medium replacement and extracellular DNases will reduce the level of free mycoplasma DNA within 1 to 2 passages.

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 and ZellShield are registered trademarks and Mycoplasma Off, PCR Clean, 10CFU, 100CFU, 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

Contamination Control Kits for PCR

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

10-0201/0501/1001	Mynox®Gold Mycoplasma Elimination Reagent	2/5/10 treatments

PCR Quantification Standards, 1 x 10^8 genomes / vial

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

See Minerva Biolabs 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

100CFU™ Sensitivity Standards, 3 vials with 100 CFU each, 2 vials negative control

103-1003	*Mycoplasma arginini*
103-2003	*Mycoplasma orale*
103-3003	*Mycoplasma gallisepticum*
103-4003	*Mycoplasma pneumoniae*
103-1103	*Mycoplasma salivarium*
103-5003	*Mycoplasma synoviae*
103-6003	*Mycoplasma fermentans*
103-7003	*Mycoplasma hyorhinis*
103-8003	*Acholeplasma laidlawii*
103-9003	*Spiroplasma citri*

PCR Clean™

15-2025/-2200/-2500	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/ 4 x 500 ml/5 l
15-2001	DNA Decontamination Reagent, Wipes in dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes, refill pack	5×50 wipes

Mycoplasma Off™

15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5 l
15-1001	Surface disinfectant wipes in dispenser box	50 wipes
15-5001	Surface disinfectant wipes, refill pack	5 x 50 wipes

ZellShield®

13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/ 3 x 50 ml

WaterShield™

15-3015/-3020/-3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	15 x 10 ml/3 x 50 ml/500 ml

PCR Cycler Validation

57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	PCR Cycler Check™ OneStep	100 reactions
57-2202	qPCR Cycler Check™	100 reactions

ConviFlex™ DNAmp Mix

191-025/-100/-250	PCR Mix with Taq polymerase for conventional and qPCR	25/100/250 reactions

SwabUp™ Lab Monitoring Kits

181-0010/-0050	Sample collection and DNA extraction	10/50 samples
182-0010/-0050	Sample collection, DNA extraction and PCR system	10/50 samples

Mycoplasma Detection Kits

Minerva Biolabs offers several rapid assays and solutions to detect cell culture contaminations.

>Features	Conventional PCR	qPCR	Polymerase included	Internal Control	Process Control	Positive Control	EP 2.6.7/JP G3 conformity
Venor®GeM Classic	✓	✗	✓* ✗	✓	✓	✓	✓
Venor®GeM Advance	✓	✗	✓	✓	✗	✓	✗
Venor®GeM OneStep	✓	✗	✓	✓	✗	✓	✗
Venor®GeM qEP	✗	✓	✓	✓	✓	✓	✓
Venor®GeM qOneStep	✗	✓	✓	✓	✗	✓	✗

* available only in US or on request.

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