

NutriCulture Mycoplasma Elimination Kit

Troubleshooting Guide

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Cat No: MEK25

Use this guide for common preparation, treatment-sequence, cytotoxicity, and recurrence problems with the NutriCulture Mycoplasma Elimination Kit. The datasheet requires separate 250x MEK1 and MEK2 stocks, sequential 1x MEK1 then 1x MEK2 treatment, no concurrent use with other antibiotics, repeated treatment cycles when needed, and confirmation of negativity after an antibiotic-free observation period.

Before You Start - Quick Checks

1. Dissolve MEK1 and MEK2 separately in 2 mL sterile PBS or sterile ddH₂O to obtain 250x stocks.
2. Aliquot the stocks to avoid repeated freeze-thaw cycles.
3. Use MEK1 and MEK2 sequentially; do not use them together.
4. Use 1x MEK1 for 3 days, then 1x MEK2 for 4 days.
5. Do not use other antibiotics such as penicillin or streptomycin during MEK treatment.
6. Test for mycoplasma at the end of each cycle and retest after at least 2 weeks without antibiotics once cultures become negative.

Problem	Most likely cause	Recommended action
Stock concentration is incorrect	MEK1 or MEK2 may not have been reconstituted to the specified 250x concentration.	Dissolve each antibiotic separately in 2 mL sterile PBS or sterile ddH ₂ O to obtain 250x stock solution.
Stock performance declines after repeated use	Repeated freeze-thaw cycles may have occurred.	Aliquot the 250x stock solutions after preparation and avoid repeated freeze-thaw cycles.
Mycoplasma remains positive after one treatment week	A single MEK1/MEK2 cycle may be insufficient.	Repeat the 3-day MEK1 plus 4-day MEK2 sequence for up to 4 consecutive weeks, testing at the end of each cycle.
MEK1 and MEK2 were added together	The required sequential treatment was not followed.	Use MEK1 first and MEK2 second; the datasheet explicitly states not to use them together.
MEK1 treatment duration is incorrect	The first stage may have been shortened or extended inconsistently.	Use 1x MEK1 for 3 days.
MEK2 treatment duration is incorrect	The second stage may have been shortened or extended inconsistently.	After MEK1, replace medium and use 1x MEK2 for 4 days.
Cell growth is affected during treatment	The cell line may be sensitive to the standard 1x concentration.	The datasheet permits lower MEK1 or MEK2 concentrations for sensitive cell lines.

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Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Other antibiotics are present in the treatment medium	Penicillin/streptomycin or other antibiotics may be used together with MEK treatment.	Use fresh medium with FBS but without additional antibiotics during MEK1 or MEK2 treatment.
Highly contaminated or resistant culture remains positive	The standard duration/concentration may not be sufficient for the difficult culture.	The datasheet states that MEK1 and MEK2 can be applied up to 2x concentration for up to 8 consecutive weeks for highly contaminated cultures or resistant strains.
Culture tests negative immediately after treatment but later becomes positive	Residual contamination may not have been detected immediately after antibiotics are removed.	After a negative result, culture cells without antibiotics for at least 2 weeks and test again to confirm mycoplasma negativity.
Recurrent contamination occurs after successful elimination	Aseptic handling or incoming cultures may reintroduce mycoplasma.	Use filtered tips, strict aseptic technique, clean laboratory coat and face mask, and quarantine incoming cell cultures until mycoplasma testing is complete.
Stock is stored longer than recommended	Prepared or lyophilized antibiotics may exceed the stated storage period.	Prepared 250x stocks can be stored at -15 to -25°C for at least 6 months; lyophilized antibiotics can be stored at -15 to -25°C for at least 12 months.
Treatment starts at inconsistent cell condition	Cell confluency may vary between treatment cycles.	Seed T25/T75 cultures and begin treatment after 16-24 hours at approximately 50-60% confluency as described.

Still having trouble? Recheck separate 250x stock preparation, aliquoting, sequential 3-day MEK1 then 4-day MEK2 treatment, absence of other antibiotics, and cycle-by-cycle testing. After the first negative result, culture without antibiotics for at least 2 weeks and retest before declaring elimination complete.