PromoKine

Mycoplasma-EX Kit

Instruction Manual

Cat.No. PK-CC91-4003
PK-CC91-4003-1

Contents

Introduction

Contamination of cell cultures by mycoplasma occurs frequently. The potential effects of mycoplasma contamination in biological products are a major problem in research, diagnostics, and biotechnological production. The effects of mycoplasma are decreased quality and quantity of products manufactured and the lack of consistency and reproducibility of research results.

Current methods for the treatment of mycoplasma in cell cultures are based on antibiotics and lead mostly to suppression or inactivation of the mycoplasma. However, antibiotic therapies in particular do not always result in a successful and permanent elimination of the contaminants. Moreover, antibiotics at active concentrations exhibit cytotoxic properties, they are capable of modifying the metabolism of eukaryotic cells and they promote the development of resistant mycoplasma strains.

Description

Mycoplasma-EX is a powerful combination of biological substances specifically eliminating mycoplasma and an antibiotic in a low active dose. Due to this powerful combination, elimination of mycoplasma is highly reliable and efficient. The initial treatment of this procedure generally leads to a complete (100%) elimination of all mycoplasma in most of the applications. If the first treatment does not completely eliminate the mycoplasma, the second step - continued treatment with antibiotics - inactivates all remaining mycoplasma. The product is used for the elimination of all kinds of *Mollicutes* and related organisms (e.g. *Mycoplasma, Acholeplasma, Spiroplasma,* and *Entomoplasma*) in cell and virus cultures. It cannot be used for the treatment of mycoplasma contamination in *Chlamydia* cultures.

Components

- Initial Treatment Reagent (1 vial [PK-CC91-4003-1] or 3 vials [PK-CC91-4003]; 500 µl each; one vial per treatment; red cap)
- Succession Treatment Reagent (3 vials [PK-CC91-4003-1] or 9 vials [PK-CC91-4003]; 500 µl each; three vials per treatment; yellow cap)

Storage Conditions

Kit components are stable during shipping at ambient temperature. Upon receipt, store at 4 - 8°C.

Shelf Life

When following our recommendations, the kit is stable until the expiration date indicated on the label.

Protocol

A. Procedure for adherent and suspension cell lines:

Step 1: Transfer 4.5 ml of pre-warmed media (37°C) in a cell culture flask (e.g. T-25) or dish.

Step 2: Add 500 µl of the Initial Treatment Reagent (vial with red cap) to the media. Mix carefully.

Step 3: Split and passage the cells as thin as possible. Add 5 ml of the cell suspension to the media containing the Initial Treatment Reagent from step 1 and step 2.

Important: The cell culture medium should contain a final FCS (fetal calf serum) concentration of 5 % (v/v). Since the serum binds and buffers the active component of the Mycoplasma-EX, it is required to add the indicated concentraiton of serum to the culture medium during the treatment Reduced serum concentrations lead to an increase of cytotoxic effects and serum deprivation may even result in cell death.

The cells should not exist as aggregates but as single cells during the treatment. Please use a microscope to confirm. To release cells from aggregates, trypsinize (if necessary) extensively or pipette cells up and down.

Step 4: Let cells grow to 80-90% confluency.

Step 5: Split the cells and passage them at the usual amount. Add 500 µl of the Succession Treatment Reagent (vial with

yellow cap) to 9.5 ml of passaged cells in fresh media. Note: Adjust the FCS concentration. Supplementation to 5% is not required any longer.

Step 6: Repeat Step 4-5 twice. After the third treatment (and a total of 4 passages starting with the initial treatment) the procedure is finished and the culture is free of mycoplasma.

B. For pre-treatment of virus stocks (before the stocks are used for cell infection), you can proceed as follows:

Step 1: 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. Dilute in a 15 ml Falcon tube 500 µl of the initial treatment reagent (red cap) with 4.5 ml medium resulting in 5 ml treatment mix. The medium should be free of fetal calf serum or other formulations containing proteins or lipids.

Step 2: Add 5 ml virus stock to the diluted reagent. The virus-containing medium should contain fetal calf serum in a concentration of 6 to 10% (v/v) or other formulations containing proteins or lipids to result in a final concentration of 3 to 5% fetal calf serum in the treatment mix.

Step 3: When the viruses have an envelope, the treatment should be stopped after 30 minutes by adding serum to a final concentration of > 10% (v/v) or four times dilution with medium.

Step 4: Perform host cell infection (duration depends on the respective virus) and change medium.

Step 5: Perform succession treatment of the cells/viruses according to the Mycoplasma-EX protocol directly to the fresh medium.

Determine Purity of Cell Culture

Mycoplasma-EX-treated cell cultures and virus stocks should be subcultivated for at least two additional passages without mycoplasma-active antibiotics and then assayed for mycoplasma recurrence to confirm successful elimination. For highly sensitive and reliable detection of mycoplasma contamination, we recommend our PCR Mycoplasma Test Kits (see below). PCR detection methods should not be used directly after treatment. Mycoplasma-EX 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. However, medium replacement and extracellular DNases will reduce the level of free mycoplasma DNA within 1 to 2 passages.
Other mycoplasma detection methods (e.g. fluorescent staining or enzymatic methods) are less sensitive and reliable and may lead to in false-negative results.

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

For convenient and sensitive detection of mycoplasma contamination, we recommend using our PCR Mycoplasma Test Kits for conventional PCR (order #: PK-CA91-1024, PK-CA91-1048, PK-CA91-1096) or Real-Time qPCR (order #: PK-CA91-3025C, PK-CA91-3050C).

For disinfecting laboratory surfaces and apparatuses such as work benches, incubators etc. we recommend using Mycoplasma-ExS Spray (order #: PK-CC91-5051), PromoCidal Spray (order #: PK-CC91-5052), or Spore-EX Spray/Wipes (order #: PK-CC91-5053 and PK-CC91-5053-W120).
For disinfecting water baths or water trays in CO_2 incubators, we recommend using Aquaguard-1 (order #: PK-CC01-867-1B) and Aquaguard-2 (order #: PK-CC01-916-500).

See our website for more information.

Ordering Information

Product Name	Size	Catalog Number
Mycoplasma-EX Kit	1 treatment	PK-CC91-4003-1
Mycoplasma-EX Kit	3 treatments	PK-CC91-4003

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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