



Mycoplasma in cell culture detection and elimination

Introduction

Surveys of cultures from labs all over the world reveal a strong prevalence of contamination by Mycoplasma and other Mollicutes. Depending on the method of detection 10-40% of continuous cell lines have been tested positively. The species most frequently found are *Mycoplasma orale*, *M. fermentans* (human), *M. arginini*, *Acholeplasma laidlawii* (bovine), and *M. hominis* (swine).

Sources of contamination

There are various possible sources for contamination by Mycoplasma. During recent years, a rising awareness of the problem may have changed the contribution of the individual sources. Culture reagents such as bovine serum have been a considerable source of contamination in the past. Today, most labs prefer Mycoplasma-free tested sera. Laboratory personnel may introduce Mycoplasma into cultures, are now trained to avoid contamination during the handling of cultures. However, other sources are even more difficult to avoid. Any addition to the culture is relevant, such as virus suspensions, antibody solutions, or media ingredients. Mycoplasma from original tissue isolates contribute to less than 1% to the reported cases. The most common source by far is cross-contamination from infected cultures. Labs exchange infected cultures and thereby inadvertently distribute Mycoplasma. PanReac AppliChem provides the tools for detection and treatment of Mycoplasma for every cell culture laboratory. For the detection by microscopy we are offering the proven fluorescent dye DAPI (product code A1001, available in pack sizes from 10 mg to 10 g).

Detection by PCR

In recent years the sensitive polymerase chain reaction (PCR) became a standard method for the detection of Mycoplasma contamination in biological samples such as mammalian cell cultures. The PCR is established in almost all life science labs either as standard PCR or real time/quantitative PCR. For your preferred setup, we offer three different kits to choose from.



Keywords

- Mycoplasma contamination
- Mycoplasma-induced cellular effects
- PCR detection of Mycoplasma
- Antibiotics for cell culture treatment

The rRNA gene sequences of prokaryotes including mycoplasmas are well conserved, whereas the lengths and sequences of the spacer region in the rRNA differ from species to species. The detection procedure utilizes the PCR for amplification of a conserved and Mycoplasma-specific 16S rRNA gene region. This system does not allow the amplification of DNA originating from other sources, such as cultured cells or bacteria, which affect the detection result. Amplification of the gene sequence with PCR using this primer set enhances not only the sensitivity, but also the specificity of detection. Amplified products are detected by agarose gel electrophoresis or by real time/quantitative PCR (qPCR Mycoplasma Test Kit, product code A9019).



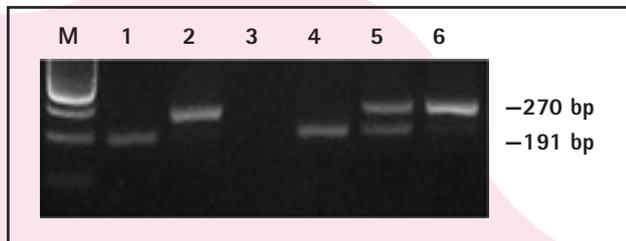
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Mycoplasma detection kits using standard PCR:

	PCR Mycoplasma Test Kit (A3744)	PCR Mycoplasma Test Kit I (A9753)	TPCR Mycoplasma Test Kit II (A8994)
Kit components:	<ul style="list-style-type: none"> Reaction Mix (PCR primers, dNTPs, Taq DNA polymerase) Buffer solution Positive template control 	<ul style="list-style-type: none"> Reaction Mix (including PCR primers, dNTPs, hot-start Taq DNA polymerase; internal amplification control) Rehydration buffer Tris buffer solution Positive template control <p><i>Kit meets criteria of section 2.6.7 of Ph.Eur.</i></p>	<ul style="list-style-type: none"> Reaction Mix (including PCR primers, dNTPs) Reaction Buffer Solution PCR grade water Positive template control Internal control DNA <p><i>Kit meets criteria of section 2.6.7 of Ph.Eur.</i></p>
Taq DNA polymerase	included	included	not included*
Form of delivery	ready-to-use master mix, liquid	single components, lyophilized	single components, lyophilized
Shipping	cooled	cooled	ambient temperature
Product codes	A3744,0020 20 tests	A9753,0025 25 tests	A8994,0025 25 tests A8994,0050 50 tests A8994,0100 100 tests

* Use kit A8994 in combination with hot-start polymerase. We recommend PanReac AppliChem SuperHot Taq DNA polymerase A5231.



Possible PCR products of PCR Mycoplasma Test Kit II:
 1: negative control; 2: positive control; 3: inhibited sample;
 4: negative sample; 5: contaminated positive sample; 6:
 contaminated positive sample with high Mycoplasma DNA
 concentration; M: DNA marker

Treatment of Mycoplasma Infections in Cell Cultures

PanReac AppliChem offers well-proven treatments to achieve reliable elimination of Mycoplasma infections from mammalian cell cultures. Precious cell cultures that are infected cannot always be simply discarded and replaced by new ones. For both, biological and economical reasons it is important to eliminate Mycoplasma from cell cultures used in basic research, diagnostics, and biotechnological production.

	Myco-1 Et-2
Application	For the treatment of all mammalian cell lines including embryonic stem cells (ES cells). Both agents are used in combination, one after another.
Components	Myco-1 (A5222), based on the antibiotic Tiamulin Myco-2 (A5233), based on the antibiotic Mino-cycline
Form of delivery	sterile 100X concentrated antibiotic solutions
Product codes	A8360,0010 1 Set (2x10 ml) A8360,0020 1 Set (2x20 ml) A8360,0100 1 Set (2x 100 ml)

	Myco-3
Application	Eliminates the most common Mycoplasma contaminants including <i>M. orale</i> , <i>M. hyorhinis</i> , <i>M. fermentans</i> , <i>M. arginini</i> , as well as <i>A. laidlawii</i> . At the concentrations recommended for use (1 µg/ml), no cytotoxic effects have been found
Components	Myco-3 is based on the antibiotic Ciprofloxacin
Form of delivery	100X concentrated antibiotic solution
Product codes	A5240,0010 10 ml A5240,0020 20 ml A5240,0100 100 ml

	Myco-4
Application	Novel combination of antibiotic and biophysical agents. For maximum efficiency and a broad spectrum. Almost 100 % of permanent eradication of Mycoplasma is achieved
Components	One kit is needed for a treatment. Each kit contains <ul style="list-style-type: none"> 1 vial of Starter Treatment solution 3 vials of Main Treatment solution
Form of delivery	sterile, ready-to-use solutions
Product codes	A8366,0002 2 Kits/Treatments

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