



## 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 mycoplasmas 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).



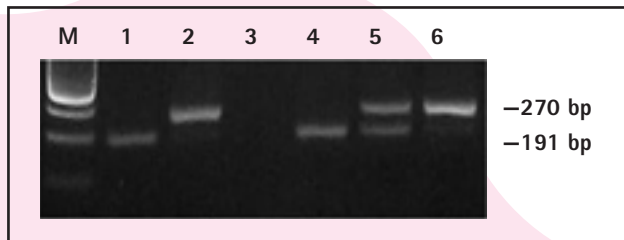
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# Mycoplasma in cell culture - detection and elimination

## Mycoplasma detection kits using standard PCR:

	PCR Mycoplasma Test Kit (A3744)	PCR Mycoplasma Test Kit II (A8994)	qPCR Mycoplasma Test Kit (A9019)
<b>Kit components:</b>	<ul style="list-style-type: none"> <li>Reaction Mix (PCR primers, dNTPs, Taq DNA polymerase)</li> <li>Buffer solution</li> <li>Positive template control</li> </ul>	<ul style="list-style-type: none"> <li>Reaction Mix (including PCR primers, dNTPs)</li> <li>Reaction Buffer Solution</li> <li>PCR grade water</li> <li>Positive template control</li> <li>Internal control DNA</li> </ul> <p><i>Kit meets criteria of section 2.6.7 of Ph.Eur.</i></p>	<ul style="list-style-type: none"> <li>Reaction Mix (including PCR primers, dNTPs)</li> <li>Reaction Buffer Solution</li> <li>PCR grade water</li> <li>Positive template control</li> <li>Internal control DNA</li> </ul>
<b>Taq DNA polymerase</b>	included	not included*	included
<b>Form of delivery</b>	ready-to-use master mix, liquid	single components, lyophilized	single components, lyophilized
<b>Storage</b>	-20 °C	2 - 8 °C	2 - 8 °C
<b>Product codes</b>	A3744,0020    20 tests	A8994,0025    25 tests A8994,0050    50 tests A8994,0100    100 tests	A9019,0025    25 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 & 2
<b>Application</b>	For the treatment of all mammalian cell lines including embryonic stem cells (ES cells). Both agents are used in combination, one after another.
<b>Components</b>	Myco-1 (A5222), based on the antibiotic Tiamulin Myco-2 (A5233), based on the antibiotic Mino-cycline
<b>Form of delivery</b>	sterile 100X concentrated antibiotic solutions
<b>Product codes</b>	A8360,0010    1 Set (2x10 ml)

	Myco-3
<b>Application</b>	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
<b>Components</b>	Myco-3 is based on the antibiotic Ciprofloxacin
<b>Form of delivery</b>	100X concentrated antibiotic solution
<b>Product codes</b>	A5240,0010    10 ml A5240,0020    20 ml A5240,0100    100 ml

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

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**AppliChem GmbH**  
Ottoweg 4  
DE-64291 Darmstadt  
Germany  
Phone +49 6151 9357-0  
Fax +49 6151 93 57-11  
[info.de@itwreagents.com](mailto:info.de@itwreagents.com)

**Nova Chimica Srl**  
Via G. Galilei, 47  
I-20092 Cinisello Balsamo  
(Milano) Italy  
Phone +39 02 66045392  
Fax +39 02 66045394  
[info.it@itwreagents.com](mailto:info.it@itwreagents.com)

**PanReac Química SLU**  
Garraf, 2  
Polígono Pla de la Bruguera  
E-08211 Castellar del Vallès  
Barcelona (Spain)  
Phone +34 937 489 400  
Fax +34 937 489 401  
[info.es@itwreagents.com](mailto:info.es@itwreagents.com)

[www.itwreagents.com](http://www.itwreagents.com)