

MYCOPLASMA- DETECTION , TREATMENT AND PREVENTION TOOLS

MYCOPLASMA PCR DETECTION KIT & ANTIBIOTIC TREATMENT



Mycoplasma Detection (Identification tools)

Mycoplasma is a prokaryotic microorganism of the class Mollicutes that lack a true cell wall, and many of which are considered pathogenic.

Mycoplasma contamination is often detected in cell cultures, and consequently, virus cultures, vaccines and other biological materials produced in cells become contaminated as well.

Mycoplasma contamination in cell lines used for research poses a serious problem. In most cases, visual detection of such contaminations or detection with the aid of a microscope is impossible. Although mycoplasma does not cause visible damage to cells, it undeniably affects cell metabolism, cell growth in culture, protein synthesis, cytokine secretion, and even causes damage to DNA and RNA. Hence, results obtained from experiments are liable to be biased when mycoplasma is present. Various studies show that the percentage of contaminated cultures in cell banks is 10%-80%. Mycoplasma contamination can originate from bovine serum, laboratory employees, other contaminated cultures, or the animals from which the cells have been harvested.

The most prevalent species of mycoplasma detected in contaminated cell cultures include *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis* and *M. pirum*.

Testing Methods

Several methods for the detection of mycoplasma have been published:

- Cultures on agar, liquid media, or semi-solid media.
- DAPI Staining - staining DNA with fluorescent dyes (4', 6-diamine-2 phenylindole dihydrochloride).
- DNA hybridization.
- Antibodies for specific mycoplasma species.
- Electronic microscope.
- PCR: specific primers.
- Biochemical - detection of mycoplasmal enzymes by colorimetric or luminescence assay.

Using PCR for the Detection

The testing required by the regulatory authorities is seeding in culture (agar and liquid media). This test is complicated, time consuming (about 5 weeks), and some mycoplasma species are difficult to detect with this method. In recent years, the disadvantages of these methods have been acknowledged (such as sensitivity, specificity and long and complex procedures), and use of PCR for the detection of contaminations in cell cultures has become increasingly widespread.

PCR has been shown to be a highly sensitive, specific and rapid method

for the detection of mycoplasma contamination in cell cultures. Specific primers have been designed from DNA that is coded to the ribosomal RNA (16SrRNA). The gene sequences for RNA are considered conserved sequences and are similar in the various mycoplasma species, which have not undergone significant mutation. Consequently, primers can be designed for these areas, which are specific to the mycoplasma and will not detect bacterial or animal DNA sequences.

The literature describes several PCR methods for the detection of mycoplasma, such as using a number of primers to obtain detection of specific mycoplasma species, and nested PCR (two consecutive PCR cycles using different primers) for amplifying sensitivity and specificity.

PCR testing techniques are based on amplification of a DNA fragment using primers prepared in advance, and fragment identification is usually carried out with electrophoresis.

In conjunction with Prof. Shlomo Rottem of the Mycoplasma Laboratory at the Hebrew University-Hadassah Medical School, Jerusalem, Biological Industries has developed the EZ-PCR Mycoplasma Test Kit (Cat. # 20-700-20; 20-700-10) a PCR-based mycoplasma test kit that simplifies testing and detection of mycoplasma contamination in cell cultures. The kit includes a unique reaction mix that contains all the ingredients required for PCR: nucleotides, primers, Taq Polymerase and magnesium. No prior preparations are required for PCR, other than the sample to be tested (centrifugation and suspension in the buffer supplied with the kit). After performing agarose gel electrophoresis, positive samples will yield a 270bp fragment. The test takes approximately five hours to complete.

The primers have been designed to detect the mycoplasma species responsible for most contaminations in cell cultures (including *Acholeplasma*). The primers were tested and found to be specific to mycoplasmatic DNA, and do not react with animal or bacterial DNA.

In sensitivity tests for the detection of defined mycoplasmas, the EZ-PCR Mycoplasma Test Kit was found to be very sensitive in comparison to other test kits currently available on the market (Table 1). The ability to routinely conduct rapid and simple tests to detect mycoplasma contamination in cell cultures facilitates the eradication or treatment of contaminated cells.



Table 1: Minimal concentration of mycoplasma detected with EZ-PCR Mycoplasma Test Kit

	Without Sample Preparation	After Sample Preparation (conc. 1/20)*
M. fermentans	240 CFU/ml	12.00 CFU/ml
M. capricolum	110 CFU/ml	5.50 CFU/ml
M. penetrans	200 CFU/ml	16.66 CFU/ml
M. hyorhinis	210 CFU/ml	10.50 CFU/ml

* According to EZ-PCR Mycoplasma Test Kit instructions.

EZ-PCR Mycoplasma Test Kit

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-PCR Mycoplasma Test Kit	20-700-10	10 assays	-20°C
	20-700-20	20 assays	-20°C

Ready-to-use PCR Mix for the detection of mycoplasma in cell culture
For complete protocol click here

EZ-PCR Mycoplasma Test Kit is designed to detect the presence of mycoplasma in contaminated biological materials, such as cultured cells. Mycoplasma detection by the direct culture procedure is time-consuming and some mycoplasma species are difficult to cultivate. With PCR testing, results are obtained within a few hours, since the presence of contaminant mycoplasma can be easily detected simply by verifying the bands of amplified DNA fragments in electrophoresis. There is no need to prepare probes labeled with radioisotopes, or to calculate enzyme, dNTPs or buffer concentrations. Instead, a ready-to-use, optimized PCR mix is supplied. The reaction mix contains a precipitant for direct loading of PCR products onto agarose gel. The primer set allows detection of various mycoplasma species (M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritis, M. bovis, M. pneumoniae, M. pirum and M. capricolum), as well as Achleoplasma and Spiroplasma species, with high sensitivity and specificity.

Advantages

EZ-PCR Mycoplasma Test kit is based on a simple assay protocol and has the following advantages:

- Highly sensitive.
- Mycoplasma-specific primers with broad range.

Convenient and user-friendly: supplied with complete reaction mix (with Taq polymerase). Requires no more than 10-20 minutes of actual work.

- Samples are easy to prepare.
- Results are easily determined with a single PCR process.
- Rapid: results obtained in no more than 5 hours.
- No loading dye needed for the agarose gel.

Kit Components

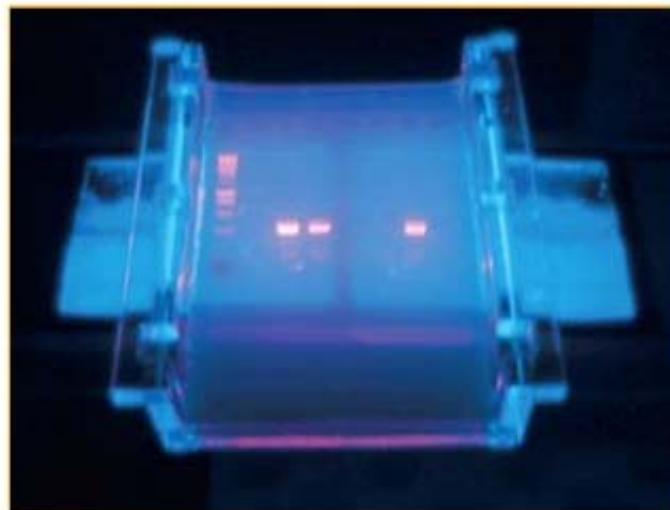
Kit Components	EZ-PCR Mycoplasma Test kit Catalogue No. 20-700-10	EZ-PCR Mycoplasma Test kit Catalogue No. 20-700-20
Reaction Mix	100µl	200 µl
Buffer Solution	0.5ml	1ml
Positive Template Control	10µl	20µl

Principle

rRNA gene sequences of prokaryotes, including mycoplasmas, are well conserved, whereas, the lengths and sequences of the spacer region in the rRNA operon (for example the region between 16S and 23S gene) differ from species to species.

The detection procedure utilizing the PCR process with this primer set consists of:

1. Amplification of a conserved and mycoplasma-specific 16S rRNA gene region using two primers.
2. Detection of the amplified fragment by agarose gel electrophoresis. This system does not allow the amplification of DNA originating from other sources, such as tissue samples 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 then detected by agarose gel electrophoresis.





Treatment of Mycoplasma-Infected Cells with Antibiotics

Using Antibiotics to Disinfect Cell Cultures

The increasingly widespread use of more sophisticated and sensitive methods for the detection of mycoplasma contamination in cell cultures has resulted in contamination being detected in numerous cultures. This raises the issue of how to eliminate mycoplasma contamination. Naturally, the ideal solution is to discard the contaminated cells. However, if the cells that are stored in liquid nitrogen are also contaminated, a solution is required for eliminating the mycoplasma and preparing a new cell bank, particularly if the cells are unique and the result of extensive work.

A number of effective methods for the elimination of mycoplasma contamination in cell cultures have been published, such as:

- Treatment with specific hyperimmune serum (antibodies).
- Passage of contaminated cells in thymus-deficient mice.
- Exposure to analogs of nucleic acids that prevent reproduction of mycoplasma.
- Treatment with antibiotics.
- Exposure of contaminated cells to mouse macrophages.
- A technique that combines growing cells on soft agar and treatment with antibiotics.

The preferred method in terms of simplicity is treatment with antibiotics, which do not damage or alter cells. Antibiotics such as penicillin, which attacks bacterial cell walls, are ineffective in this instance, since mycoplasma lacks a true cell wall. Several antibiotics eliminate mycoplasma effectively, such as Tylosin, Neomycin, Tetracycline and Gentamycin. However, the efficacy of these antibiotics is restricted to specific mycoplasma species and frequently only reduces the concentration of mycoplasmas, rather than disinfect the cell culture. Consequently, as soon as treatment is concluded, contamination will recur.

Two methods are recommended for treating contaminated cells with antibiotics. The first is based on alternating treatment with two types of antibiotics (Tiamutin and Minocycline), and the second on treatment with one type of antibiotic (Ciprofloxacin).

Summary

Heightened awareness regarding mycoplasma contamination, and increased use of sensitive and effective methods for the detection and treatment of mycoplasma contaminations, will lead to a reduction in the percentage of contaminated cultures. In addition to isolating contaminated cultures, and discarding or treating them, meticulous work procedures should be followed, and only mycoplasma-free raw materials should be used.

The contamination of cells with mycoplasma is a very common problem, although it often goes unnoticed since no cloudiness appears in the cell culture. Nevertheless, the contamination often causes biochemical changes as well as changes in the immunological properties of the cells. Since mycoplasma-infected cells cannot always be discarded, many complex methods have been suggested for the elimination of mycoplasma.

Biological Industries is now offering a combination of antibiotics, which have been shown to be effective in the elimination of mycoplasma species that account for 90% of the contamination found in cell cultures.

When used according to the following instructions, no cytotoxic effects will occur.

BIOMYC-1 & BIOMYC-2

Product Name	Catalogue Unit No.	Unit Size	Storage Temp.
BIOMYC-1 Antibiotic Solution 100X Conc.	03-036-1B	100ml	-20°C
	03-036-1C	20ml	-20°C
	03-036-1D	10ml	-20°C
BIOMYC-2 Antibiotic Solution 100X Conc.	03-037-1B	100ml	-20°C
	03-037-1C	20ml	-20°C
	03-037-1D	10ml	-20°C

BIOMYC-1 is based on the antibiotic Tiamutin, which is produced by the fungus *pleurotus mutilus*. BIOMYC-2 is based on minocycline, which is a tetracycline derivative. These two antibiotic solutions are generally used sequentially in combination.

Instructions for Use

1. Do not use the two solutions together, but sequentially.
2. Add 1ml BIOMYC-1 to 100ml medium, and maintain the contaminated cells in this mixture for 4 days. Any fresh medium added should also contain BIOMYC-1.
3. After 4 days, add 1ml BIOMYC-2 to 100ml fresh medium, and maintain the cells in this second mixture for 3 days.
4. The above, together, are considered as one treatment cycle. It may be necessary to repeat this cycle 2-3 times.
5. During the process, the cells can be tested for mycoplasma contamination, and results can then be used to shorten the process when possible.





BIOMYC-3

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOMYC-3 Antibiotic Solution 100X Conc.	03-038-1B	100ml	-20°C
	03-038-1C	20ml	-20°C
	03-038-1D	10ml	-20°C

BIOMYC-3 is based on the ciprofloxacin antibiotic, which is a member of the fluoroquinolone group. Many mycoplasma species have been found to be sensitive to BIOMYC-3, including *A.laidlawii*, *M. orale*, *M. hyorhinis*, *M. fermentans* and *M. arginini*. These species are responsible for most of the contamination in cell culture⁽¹⁾. At the recommended concentrations, no cytotoxic effects have been found, and the treatment is quite easy to perform.

Instructions for Use

1. Add 1ml BIOMYC-3 to 100ml medium.
2. Continue the treatment for a total of 14 days, changing the medium (containing BIOMYC-3) every 2-3 days.
3. Retain the cells in the growth medium for an additional 14 days before re-testing for mycoplasma.

(1) Schmitt, k. et al., J. Immunol. Methods, 109: 17-25 (1988)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Mycoplasma Detection			
EZ-PCR Mycoplasma Test Kit	20-700-10	10 assays	-20°C
	20-700-20	20 assays	-20°C
Treatment of Mycoplasma-Infected Cells with Antibiotics			
BIOMYC-1 Antibiotic Solution 100X Conc.	03-036-1B	100ml	-20°C
	03-036-1C	20ml	-20°C
	03-036-1D	10ml	-20°C
BIOMYC-2 Antibiotic Solution 100X Conc.	03-037-1B	100ml	-20°C
	03-037-1C	20ml	-20°C
	03-037-1D	10ml	-20°C
BIOMYC-3 Antibiotic Solution 100X Conc.	03-038-1B	100ml	-20°C
	03-038-1C	20ml	-20°C
	03-038-1D	10ml	-20°C