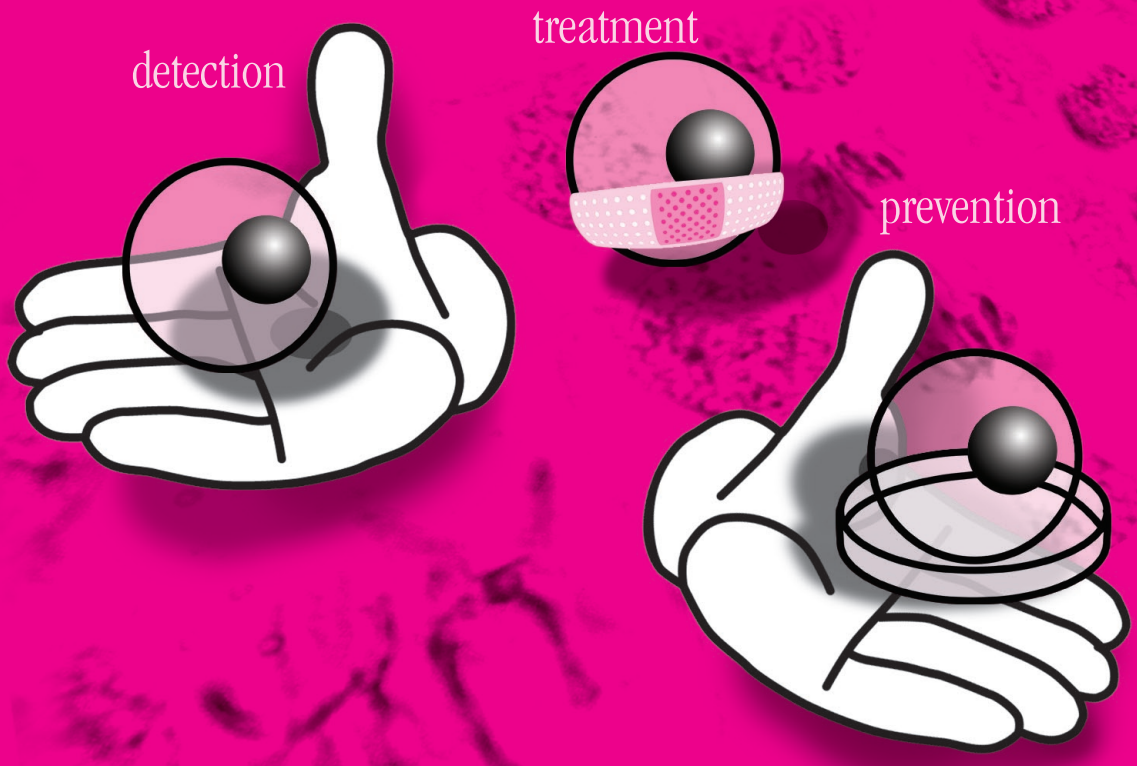
# Safety First: Banish Mycoplasma.

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detection

# PCR Mycoplasma Test Kit A3744

## *Ready-to-use* PCR Mix for the detection of Mycoplasma in Cell Culture

The PCR Mycoplasma Test Kit is designed to detect the presence of mycoplasma contaminating 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, dNTP's or buffer concentrations. Instead *ready-to-use*, optimized PCR mix is supplied. The primer set allows detection of various mycoplasma species (*M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. bovis, M. pneumoniae, M. pirum* and *M. capricolum*), as well as Acholeplasma and Spiroplasma species, with high sensitivity and specificity.

Knowledge is good –
Checking is better:
The PCR Mycoplasma Test Kit detects Mycoplasma contamination in cell cultures. The results are obtain within a few hours: simple, quick and reliable.

## Kit Components

| | |
|---|---|
| 1. Reaction Mix | 200 µl |
| 2. Buffer Solution | 1 ml |
| 3. Positive Template Control | 20 µl |

## Reagents not supplied in the kit

1. Mineral Oil (Product-No.: A3920)
2. Agarose gel (see catalog for agaroses)
3. Distilled sterilized water (Product-No.: A4042)

## Equipment required

- Authorized thermal cycler for PCR
- Microcentrifuge tubes
- Agarose gel electrophoresis apparatus
- Microcentrifuge
- Micropipets and pipette tips (autoclaved)

## Storage -20°C

Avoid repeated changes in the Reaction Mix temperature. When in use, always keep the Reaction Mix on ice!

## Reference

Rottem, S. & Barile, F.M. (1993) *TIBTECH* **11**, 143-150

## 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 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 then detected by agarose gel electrophoresis.

## Protocol

### A. Test sample preparation

Transfer 0.5 – 1.0 ml cell culture supernatant into a 2 ml centrifuge tube. To pellet cellular debris, centrifuge the sample at 250xg briefly. Transfer the supernatant into a fresh sterile tube and centrifuge at 15,000 – 20,000 xg for 10 minutes to sediment mycoplasma. Carefully decant the supernatant and keep the pellet (the pellet will not always be visible). Re-suspend the pellet with 50 µl of the Buffer Solution and mix thoroughly with a micropipet. Heat to 95°C for 3 minutes. The test sample can be stored at this stage at -20°C for later use.

### B. PCR amplification

1. Prepare the reaction mixture in a PCR tube by combining the reagents shown below:

| Reagents | Volume |
|---|---|
| $H_2O$ | 35 µl |
| Reaction Mix | 10 µl |
| Test sample | 5 µl |

2. Overlay mineral oil (approximately 40 µl) to avoid the evaporation of the reaction mixture.
3. Place all tubes in DNA thermal cycler. Set the parameters for the following conditions and perform PCR.

| | | |
|---|---|---|
| 94°C | 30 secs. | |
| 94°C | 30 secs. | 35 cycles |
| 60°C | 120 secs. | |
| 72°C | 60 secs. | |
| 94°C | 30 secs. | |
| 60°C | 120 secs. | |
| 72°C | 5 min. | |

**C. Analysis of amplified products by gel electrophoresis**

1. Apply 20 µl of the PCR product to the gel electrophoresis.
2. Perform agarose gel electrophoresis with the PCR amplified samples to verify the amplified product and its size. Use 2 % agarose gel. The size of DNA fragments amplified using the specific primers in this kit is 270 bp.

**D. Control Template**

By the use of 1 µl of Positive Template Control as a test sample, PCR efficiency can be checked. The size of the PCR product obtained using the positive template with primer pairs is 270 bp.

# Treatment of Mycoplasma-infected Cells with Antibiotics

## Myco-1 (A5222), Myco-2 (A5233) and Myco-3 (A5240)

Treatment…for those cases where it happened: antibiotics are the effective treatment for Mycoplasma contamination.

The contamination of cells with mycoplasma is a very common problem, even though 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 complicated methods have been suggested for the elimination of the mycoplasma.
AppliChem 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 culture. When used according to the following instructions, no cytotoxic effects will occur.
Store Myco solutions at –20°C.


### Myco-1 (A5222) and Myco-2 (A5233)

Myco-1 is based on the antibiotic Tiamulin, which is produced by the fungus *Pleurotus mutilus*.
Myco-2 is based on Minocycline, a Tetracycline derivative.
Myco-1 (A5222) and Myco-2 are generally used sequentially in combination.

**Instructions for use**

**1.** Do not use the two solutions together, rather sequentially!
**2.** Add 1 ml Myco-1 to 100 ml medium, and maintain the contaminated cells in this mixture for 4 days.
Any fresh medium added should also contain Myco-1.
**3.** After 4 days, add 1 ml Myco-2 to 100 ml 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.

### Myco-3 (A5240)

Myco-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 Myco-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. At the concentrations recommended for use, no cytotoxic effects have been found, and the treatment is quite easy to perform.

**Instructions for use**

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

**Literature**

Schmitt, K. *et al.* (1988) *J. Immunol. Methods* **109**, 17-25

treatment

# Disinfecting $CO_2$-Incubators and Water Baths

## Disinfectant Solutions for Incubators and Sterile Benches in Cell Culture and Molecular Biology Laboratories

prevention

### Incubator-Clean (A5230)

The problem of contamination in incubators and/or sterile work-benches is often a serious one, leading to extensive damage. Incubator-Clean solution prevents contamination with and growth of fungi (and spores), bacteria (and spores), mycoplasma and viruses (including HIV and Hepatitis B). The active ingredients are quaternary benzylammonium compounds, and the solution does not contain mercury, formaldehyde, phenol or alcohol. Furthermore, Incubator-Clean is non-toxic and biodegradable. It was also found to be fully compatible with common work surfaces. Incubator-Clean is supplied in spray bottles.

Don't let it happen: Incubator-Clean – non-toxic and biodegradable – in a practical spray bottle.
With Incuwater-Clean, you can finally say goodbye to contaminated water in your $CO_2$ incubator.
Aquabator-Clean for the disinfection of standard water baths – non-toxic and biodegradable, of course.


### Recommended use

Spray incubators once every 2 weeks. It is not necessary to empty the incubator before spraying.
Spray sterile benches once a day, or preferably before each laboratory worker begins to use the work area. The drying time is the reaction time!

### Incuwater-Clean (A5219)

The water required to create the humidity is a source of contamination which disperses in the incubator. In order to disinfect the water we recommend Incuwater-Clean, which contains a disinfectant that does not cause damage to the stainless steel tray, is non-toxic, non-volatile, and ex tremely effective. Incuwater-Clean is supplied as a 100X concentrated *ready-to-use*-solution (100 ml).
**Caution:** For disinfecting "normal" water baths, use the product Aquabator-Clean (A5225)!
Aquabator-Clean is not suited for use in $CO_2$-incubators!)

### Recommended use

The water should be replaced with sterile water every two to four weeks, adding 50 ml of Incuwater-Clean per 5 liters of water.

### Aquabator-Clean (A5225)

Aquabator-Clean is intended for disinfecting various kinds of water baths from bacteria and fungi. It is recommended to use 2 ml Aquabator-Clean for each liter of water in the bath, and to repeat the procedure every 1 – 2 weeks. After 4 – 6 weeks, the bath should be emptied and refilled with water containing Aquabator-Clean. The active ingredient in Aquabator-Clean is safe to humans and does not cause any irritating effects to the skin when used in the recommended concentration. It is biodegradable.
**Caution:** Aquabator-Clean is not suited for use in $CO_2$-incubators!

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