Safety First:
Banish Mycoplasma

detection  treatment  prevention

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Firstly reported in 1956 (Robinson et al. 1956), the potential presence of mycoplasma in cell culture laboratories continues to challenge scientists. The parasitic mycoplasmas represent a serious problem for all cell line-related fields in research as well as in industrial facilities for development or manufacture of cell-derived biological and pharmaceutical products, including vaccines, monoclonal antibodies, drugs, and products for gene and cell therapy. Still, there is no perceivable reduction of cell culture infection rates (Ryan 2008), even though risks and consequences caused by mycoplasma infections have been known for decades, and strategies for their prevention, detection and elimination are well established. Why are so many cell lines – while commonly well fostered by their cell culturists – still insufficiently protected against the cell wall-free invader? Is this due to carelessness, or rather a lack of knowledge? Unfortunately we cannot provide any data regarding this question – but a lot of facts demonstrating the importance of this unpopular subject.
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Introduction

How do mycoplasmas commonly enter our labs and cultures?

Mycoplasmas are omnipresent; their broad range of hosts includes humans and other mammals, birds, reptiles, fish, insects and plants (Razin et al. 1998). However, in cell culture laboratories, 95 % of all continuous cell line infections are caused by only six species from bovine (M. arginini & Acholeplasma laidlawii), swine (M. hyorhinis) – and human (M. orale, M. fermentans, M. hominis) origin (Drexler & Uphoff 2002). The main source of mycoplasma contaminations today are mycoplasma-infected cell cultures used in the same laboratory (Rottem & Barile 1993, Drexler et al. 2002; Drexler & Uphoff 2002). The infection may be transferred by aerosols, particulates and inadequate cell culture technique directly – or indirectly via media, solutions and laboratory equipment contaminated by previous use in processing mycoplasma-infected cells. As a result, 15 – 35 % of all continuous cell lines are positive for mycoplasma, but only 1 % of the primary cell cultures (Drexler & Uphoff 2002). The second leading source is the laboratory personnel, explaining the fact that mycoplasma species from bovine origin or swine were traced back to contaminated sera and other animal-originating products, e.g. the prevalent presence of A. laidlawii and M. arginini indicating fetal or newborn bovine serum as the primary source of infection.

Nowadays, sera and media are rarely the source of mycoplasma contamination (Lincoln & Lundin 1990; Armstrong et al. 2009) as long as they are purchased from reputable manufacturers that sterilize their products by several filtration steps using a 0.1 µm pore membrane filter and frequently control sterility.

What makes mycoplasma species worse than other bacterial contaminates – and why is it a must to banish them from cell cultures?

In contrast to “common” bacteria, these tiny prokaryotes do not possess a cell wall. Together with other cell wall-lacking bacteria – species of ureaplasma, acbioleplasma, anaeroplasma, spiroplasma – they form the class of mollicutes. Nevertheless, the terms “mycoplasma” or formerly “pleuropneumonia-like organisms (PPLO)” and “mollicutes” are often used synonymously. Due to the absence of a cell wall, mycoplasmas are unaffected by antibiotics that interfere with peptidoglycan formation, namely beta-lactam antibiotics. These include penicillin-derivatives, cephalosporins, and carbapenemes. Furthermore they are very flexible in shape which in addition to their small size (ranging from 0.1 to 0.8 µm in diameter, depending on the literature) makes them difficult to filter from solutions. Mycoplasma species easily penetrate the membrane of 0.2 µm filters commonly used for sterilization of media, sera and other non-autoclavable reagents. Mycoplasma’s general dependence on complex enriched media (including host cell nutrients) and defined environmental conditions – both perfectly realized in cell culture – and their very slow growth rates complicate identification of infected cells by common microbiological cultivation methods. Their small size and missing cell wall allows them to achieve high densities in cell cultures; often without being detectable by turbidity, cytopathogenicity or even microscopic examination. However, the consequences of mycoplasma contaminations should not be underestimated; neither with regard to research (and the researcher’s career!), nor in terms of serious health risks for humans and animals. Please keep in mind that some members of the mycoplasma family are pathogenic organisms!

By growing covertly and undisturbed within a cell culture, mycoplasma can easily take over the control of reagents, equipment, and other cell lines within weeks (McGarrity 1976). Be aware that the lack of visible effects provides a false sense of security: While often behaving inconspicuous at first glance, the fastidious organisms are able to influence nearly every single cellular function, ranging from a decelerated growth rate to metabolic (including protein, RNA, DNA synthesis) and morphologic changes. All these effects are mainly based on a competition for essential nutrients (nucleosides, nucleotides, nucleobases, arginine and other amino acids, fatty acids, sugars, etc.) and the release of toxic, cytolytic or acidic metabolites. By up- and down-regulation of cytokines and growth factors, stress-response genes, transport proteins, receptors, ion channels, oxidases, tumor suppressor and oncogenes, mycoplasmas significantly alter the gene expression profiles of cultured cells (Miller et al. 2003). Therefore they make any experiment carried out with infected cells questionable! Furthermore they are known to cause chromosomal aberrations in vitro, with chromosomal breakage, translocation events, and reduction or augmentation in chromosome number being the most frequent outcomes. Virus propagation might also be influenced in both directions, positively (by inhibiting interferon induction and activity) as well as negatively (by competing for essential nutrients). Even though there are a large number of potential effects described in literature it is unpredictable which effect will occur. Possible effects depend on mycoplasma species and strain, the infected cell type, and certainly on environmental conditions (Rottem & Barile 1993).
Finally, besides biosafety concerns, the consequences of mycoplasma contamination on laboratory work are loss of time, efforts, money (regarding cells, media, materials, but also valuable biopharmaceuticals, if cultures were used for production of vaccines, antibodies or drugs) and good reputation. Research based on mycoplasma-contaminated cell lines will produce inaccurate or erroneous results yielding misleading publications. Consider the personal embarrassment and maybe the loss of good reputation, if the published results are proven to be faulty due to a contamination problem. And how awkward will it be to get informed by a colleague that the cell line you provided is contaminated? All these factors should be reconsidered, when risking covert mycoplasma infections by NOT testing cell cultures and NOT actively fighting them by good laboratory practice.

To sum up

A mycoplasma-free cell culture is a PRECONDITION for safety and purity of cell-derived products and reliable results in scientific experiments.

Prevention

How can I avoid contaminations?!

Probably, there will never be a point in time when mycoplasma contaminations are completely banished from our labs – as long as humans are working there. But carrying out some general principles will minimize the risk of contaminations and prevent costly or embarrassing situations.

- **Strictly follow aseptic techniques and practices**, including no unnecessary talking, no mouth pipetting, no media supply by pouring, regular hand washing and disinfection! Do not use the laminar flow for storage of solutions and equipment! Only work with ONE cell line at a time and use separate materials for each cell line to avoid cross-contaminations! Make sure all media, solutions and materials are properly sterilized – the same holds true for any kind of occurring waste of course!

- **Clean and disinfect** surfaces, laminar flows, incubators, water baths and all other equipment frequently – **before AND after the working procedure**. Make sure the laboratory is cleaned up regularly and only authorized persons have access to the working area.

- **Use antibiotics responsibly**. For routine culture work antibiotic-free media should be employed. General usage of antibiotics to mask low hygiene levels, a lack of good aseptic techniques, or improper cell culture facilities are not a solution to the problem! Quite the contrary, non-responsible use of antibiotics will make the situation even worse.

- **Isolate incoming cell cultures** (use a separate incubator or at least sealed flasks as well as separate culture media and materials) until the mycoplasma test results are proven to be negative.

- **Test frequently for contamination** – regardless of whether the cell culture contains any antibiotics or not! **Routine testings for the presence of mycoplasma species are an absolute must for** the responsible scientist! Only by identification and treatment or elimination of the infected cell line the risk of further (cross-) contaminations is banished and experiments yield stable and reliable results.

AppliChem offers a series of products preventing microbial contaminations – in hoods, incubators, water baths and cell cultures. More information about this product line is available on page 4 and in the product table on page 12.
Furthermore, it is highly recommended to freeze a cell stock as a backup for damaged or lost cell cultures. When dealing with cells of limited life span, cryopreservation is invaluable anyway. But also a stock of continuous cell lines should be stored properly below –130 °C to prevent *in vitro* cellular alteration (Hughes et al. 2007; Stacey & Masters 2008) and maintain reliable cultures of consistent quality for research and biopharmaceutical production. The advantages of a cryopreserved cell bank are a reduced risk of (cross-)contamination with microorganisms or other cell lines, prevention of phenotypic or genotypic drifts, and damages due to cell aging. But caution: Please be aware that mycoplasmas are able to survive freezing in liquid nitrogen – even without cryopreservation. For that reason, a contaminated liquid nitrogen container (e.g. due to an inadequately closed or contaminated sample of cells) might be a source of mycoplasma. But how do they enter the cell culture-containing cryo tube? Storage in the liquid phase together with a non-sufficient tube filling level might be the way in. Liquid nitrogen has the tendency to permeate the cryo tube, especially if it is filled insufficiently. To avoid any contamination risk, cryogenic vials should be properly stored in the vapor phase of the liquid nitrogen container.

**Disinfecting CO₂ incubators and water baths**

**Incubator-Clean™ (A5230)**
Incubator-Clean™ is a non-toxic and non-hazardous solution for disinfection of incubators, sterile benches and other surfaces in cell culture and molecular biology laboratories. The problem of contamination in incubators and/or sterile work-benches is often a serious one, leading to extensive damage. Incubator-Clean™ prevents contamination with and growth of mycoplasma and other bacteria (and spores), fungi (and spores), 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 biodegradable and fully compatible with common work surfaces. Incubator-Clean™ is supplied in spray bottles.

**Recommended use:** Spray incubators every other week. It is not necessary to clear 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. Incuwater-Clean™ does not contain heavy metals, it is non-toxic, non-volatile, and extremely effective. Incuwater-Clean™ is supplied as a 100X concentrated ready-to-use solution. The water should be replaced every two to four weeks.

**Aquabator-Clean™, 100X (A9390)**
Aquabator-Clean™ is intended for disinfecting various kinds of water baths from bacteria and fungi. The active ingredient in Aquabator-Clean™ is safe to humans and does not cause any irritating effects to the skin when used at the recommended concentration. It is biodegradable. Use 10 ml Aquabator-Clean™ for each liter of water in the bath.

**Caution:** Aquabator-Clean™ is not suited for use in CO₂ incubators!

**Preventing microbial growth in cell cultures**

**CellCultureGuard (A8906)**
Our first choice cell culture reagent!
The main measures to avoid contamination of cell cultures are sterile techniques, consistent compliance with good laboratory practice and sterilized equipment and vessels. However, for some cell lines like primary ones, hybridomas, or cell lines isolated from patients this might not be sufficient. The addition of an antibiotic is required to further reduce the risk of microbial contaminations. CellCultureGuard is a combination of novel antibiotics perfectly suited for protection of animal and human cell cultures from contamination by a wide range of microorganisms: *extra- and intracellular bacteria, mycoplasma, protozoa and fungi (yeast)*. The combination of innovative antibiotics is blocking the bacterial DNA synthesis and the protein biosynthesis. Due to the combined activity there is a very low probability for the formation of resitants. Additionally, it is highly compatible with resistance markers and, at the recommended concentration, no side effects on cell cultures are observed.

**Recommended use:** CellCultureGuard is provided as a 100-fold concentrated sterile solution. Simply add 1 ml of CellCultureGuard to 100 ml of cell culture medium (or human or animal cell cultures). To ensure maximum protection at 37 °C, CellCultureGuard should be renewed after 7 days.
How can I identify a mycoplasma contamination?

The most sensitive method to detect mycoplasma is the direct culture method in suitable broth and agar media to obtain visible colonies. Theoretically, a single CFU (colony forming unit) per sample volume is detectable. Unfortunately this method is also the most time consuming (up to 28 days; due to the slow growth of mycoplasma species), and it requires experienced personnel conducting the experiments under controlled environmental conditions. Even if the difficult procedure is properly conducted (to start with the complex medium composition often requiring non-standard adjustments for individual species up to analysis of the results), the method is not 100% effective, since some fastidious strains may not grow in pure culture. Therefore, an indirect detection method should be performed in addition. The most sensitive indirect mycoplasma tests are based on DNA fluorochrome staining (e.g. using DAPI) and PCR. Even if the detection limit of these methods is lower than for the direct culture method, they are absolutely sufficient for routine testings. Commonly, mycoplasma-contaminated cell cultures show high densities of mycoplasma (up to $10^7 – 10^8$ CFU/ml) that are well suitable for the detection limits of these methods. In contrast to the PCR alternative, the traditional fluorescence staining method requires more time and experience. In addition, the DNA-binding fluorescent stain is carcinogenic and needs to be handled carefully. Hence, for routine mycoplasma screenings, PCR analysis is recommended (Drexler & Uphoff 2002). This method is sensitive (depending on the kit almost as sensitive as the direct culture method), very fast (results are obtained within hours) and detects cultivable as well as non-cultivable mycoplasma species. Furthermore, at least with commercially available kits, this method is very easy to perform and does not require a specific expertise.

Using PCR for fast & reliable mycoplasma detection

PCR Mycoplasma Test Kits: How do they work?!

The principle is simple: A mycoplasma-specific DNA-fragment is amplified exclusively by PCR, using a specific primer set. Only if mycoplasma DNA is present in the sample, a mycoplasma-specific PCR product of defined size (270 bp) is obtained – detectable on an agarose gel, or, in case of the qPCR Test Kit, using the FAM™ detection channel. The primer set binds selectively to the highly conserved 16S rRNA operon coding region of the mycoplasma genome, not detecting any other bacterial or eukaryotic DNA. The detection range includes the most frequently occurring contaminants *M. fermentans*, *M. arginini*, *M. orale*, *M. hyorhinis*, *M. salivarium*, *M. bovis*, but also less prevalent mycoplasma species, such as *M. pneumoniae*, *Acholeplasma laidlawii*, *M. synoviae*, *M. pulmonis*, *M. bovis* – just to name a few.

A new generation of PCR Mycoplasma Test Kits

Besides a Positive Control DNA, the “new generation kits” PCR Mycoplasma Test Kit II (A8994) and qPCR Mycoplasma Test Kit (A9019), offer an optional Internal Control DNA as PCR inhibition marker. The Internal Control DNA is added to the master mix to visualize incomplete or inhibited PCR runs.

1. No mycoplasma DNA: Negative Control

   Internal Control DNA  
   Amplification

   189 bp

2. Low concentration of Mycoplasma DNA: Contaminated sample

   Mycoplasma DNA  
   Internal Control DNA  
   Amplification

3. Positive Control / Strongly contaminated sample

   Mycoplasma DNA  
   Internal Control DNA  
   Amplification

4. PCR inhibition

   ?

As a consequence, the resulting band patterns (or signals when using the qPCR Kit) allow a clear and provable evaluation: The presence of the mycoplasma-characteristic PCR product (270 bp) indicates a mycoplasma contamination, regardless of whether there is an additional band of 191 bp or not. In contrast, a single band of 191 bp correlates to a mycoplasma-negative sample. False-negative samples are no longer possible, because the lack of a band does not necessarily indicates a lack of mycoplasma DNA and therefore does not exclude the possibility of PCR inhibition.

Why should the PCR reaction not work? Especially the sample bears a risk of introducing PCR inhibiting substances into the reaction mixture. Considering the fact that the sample DNA is commonly provided as cell culture supernatant, residual cells or cell debris are the most likely reasons for PCR failure. However, phosphate containing buffer or inhibitory substances accumulating in older cell cultures may inactivate the DNA polymerase as well. The solution: Simply perform a DNA extraction prior to the PCR analysis. Standard Kits for genomic DNA isolation (e.g. A5185) are well suited for this procedure. Make sure that the purified DNA is free of alcohol.

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### Standard PCR: PCR Mycoplasma Test Kit

**Kit components**
- Reaction mix (dNTPs, PCR primers, Taq DNA polymerase)
- Buffer solution
- Positive Control DNA

**Taq DNA polymerase**
- Included

**Form of delivery**
- Ready-to-use master mix, liquid

**Shipping**
- Cooled

**Catalog numbers**
- A3744,0020 20 tests
- A8994,0025 25 tests
- A8994,0050 50 tests
- A8994,0100 100 tests

### PCR Mycoplasma Test Kit II

**Kit components**
- Reaction mix (dNTPs, PCR primers)
- PCR grade water
- Buffer solution
- Positive Control DNA
- Internal Control DNA

This kit meets criteria of Ph. Eur. section 2.6.7

**Taq DNA polymerase**
- Not included, we recommend a Hotstart Taq polymerase, e.g. A5231

**Form of delivery**
- Single components, lyophilized

**Shipping**
- Ambient temperature

**Catalog numbers**
- A8994,0025 25 tests
- A8994,0050 50 tests
- A8994,0100 100 tests

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### Real time/qPCR: qPCR Mycoplasma Test Kit

**This qPCR Mycoplasma Test Kit is suited for all common qPCR cyclers employing the ROX™ (Internal Control DNA) and FAM™ (Mycoplasma DNA) channels, e.g. LightCycler® 1.2, 1.5, 2.0, 480, Rotor-Gene™ 3000, 6000, ABI Prism® 7000, iCycler iQ®, iQ™5, Opticon 2, Chromo 4, MX300P®, MX4000®.**

**Kit components**
- Reaction mix (dNTPs, primers, probes)
- Positive Template Control
- Buffer solution
- PCR grade water
- Internal Control DNA

**Taq DNA polymerase**
- Not included, we recommend a Hotstart Taq polymerase, e.g. A5231

**Form of delivery**
- Single components, lyophilized

**Shipping**
- Ambient temperature

**Catalog numbers**
- A9019,0025 25 tests
- A9019,0100 100 tests
What can I do to eliminate mycoplasma from an infected cell culture?

This answer is easy: Autoclave the contaminated cells, at best, together with any bottle of medium and reagent used with this relevant culture. Don’t forget subsequent cleaning and disinfection of surfaces, hoods, incubators, pipettors etc. – or better, the whole lab! Make sure that other cell lines are not infected as well! Cleaning up a contaminated culture with an anti-mycoplasma treatment is recommended only for very valuable or irreplaceable cultures, and if the potential source of mycoplasma was previously banished from the laboratory. Efforts for the attempted rescue are high and, until now, no universal mycoplasma-eliminating reagent is available. Antibiotic resistance, cytotoxicity, and a reduced viability of chronically or multiply infected cells may be reasons to prevent curing (Fleckenstein & Drexler 1996).

Despite existing resistances, the most reliable and efficient treatment of mycoplasma contaminations is the addition of suitable antibiotics, such as quinolones, tetracyclines and macrolides (Drexler & Uphoff 2002). In an experiment with 251 chronically mycoplasma-positive cell lines, treatment with ciprofloxacin provided recovery-levels of 78%, with 15% of the cell cultures remaining contaminated due to resistance and 7% loss by cell death during the elimination procedure. The combination of tiamulin and minocycline even reached curing of 82% of all treated cell cultures, showing a lower resistance level (7%) but higher cytotoxicity (11% of the cell cultures died during the treatment; data taken from Drexler & Uphoff 2002). Besides the traditional mycoplasma-eliminating agents Myco-1 & 2 (tiamulin and minocycline) and Myco-3 (ciprofloxacin), AppliChem now offers a new solution for effective and permanent removal of mycoplasma species from cell culture: Myco-4 provides a broad spectrum of activity (including any type of mycoplasma, acholeplasma, spiroplasma and entomoplasma) combined with very low cytotoxicity and a low resistance risk due to an initial biophysical mode of action.

Treating mycoplasma-infected cells

...for those cases when it happened:
Antibiotics are the most effective treatment for mycoplasma contaminations!

Myco-1 & 2 Set (A8360)
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 (A5233) are generally used sequentially in combination.

Instructions for use
1. Add 1 ml Myco-1 to 100 ml medium, and maintain the contaminated cells in this mixture for 4 days.
2. After 4 days, add 1 ml Myco-2 to 100 ml fresh medium, and maintain the cells in this second mixture for 3 days.
3. The above, together, are considered as one treatment cycle. It may be necessary to repeat this cycle 2 – 3 times.

AppliChem’s PCR Mycoplasma Test Kits enable identification of mycoplasma-contaminated cell cultures – fast and effective! The PCR technique allows highly sensitive detection of both cultivable and non-cultivable mycoplasma species. Reproducible results are provided within hours, making PCR the method of choice for frequent routine testings.
AppliChem’s Myco-1 & 2, and Myco-3 for well established treatment of mycoplasma-infected cell cultures. Studies show an efficiency rate of 80% for these traditional anti-mycoplasma antibiotics.

Myco-3 (A5240)

Myco-3 is based on the antibiotic ciprofloxacin, 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, and maintain the contaminated cells in this mixture for 2–3 days.
2. Continue the treatment for a total of 14 days, while changing the medium containing Myco-3 every 2–3 days.

Myco-4 (A8366)

Myco-4 is a highly efficient combination of standard antibiotics and a biophysical agent. The biophysical agent integrates into the mycoplasma membrane and compromises its integrity. By combination with standard antibiotics, the effective dose of both, biophysical agent and antibiotics, can be reduced to a minimum for lowest cytotoxicity, still causing a highly reliable and definite elimination of mycoplasma. In addition, the biophysical properties of Myco-4 make the development of resistant strains very unlikely.

One application includes 4 vials, a *Starter Treatment* (red cap) and three *Main Treatments* (yellow cap) solutions. The *Starter Treatment* kills most of the mycoplasma particles without harming the cells. The *Main Treatment* kills the remaining particles, leading to a permanent eradication with efficiency rates of up to 100%. Each component is a sterile, ready-to-use solution.

Contaminated cells are incubated once with the *Starter Treatment* solution for the common time period of a normal passage, or at least 30 min and three times with the Myco-4 *Main Treatment* reagent (each time for the time period of a passage). Precondition for successful mycoplasma elimination is a limited number of single cells and a reduced concentration of serum during incubation with the *Starter Treatment* solution!

Instructions for use

1. Add 500 µl Myco-4 *Starter Treatment* to 4.5 ml standard cell culture medium (max. 5% v/v FCS) and transfer 5 ml of cell culture (10^4–10^5 single cells; medium with max. 5% FCS) into the mix. Incubate cells for at least 30 min up to the common time period of a normal passage.
2. Add 500 µl of the Myco-4 *Main Treatment Reagent* (vial with yellow cap) to 9.5 ml of passaged cells in fresh media. Grow the cells to 80–90% confluency, split the cells and passage at the usual rate.
3. Repeat step 2 twice.

After the third treatment and a total of 4 passages starting with the *Starter Treatment solution* the procedure is finished and the culture is free of mycoplasma according to our experience.
### Myco-1 & 2 Set

**Application**
For the treatment of all mammalian cell lines including embryonic stem cells (ES cells).

**Components**
- Myco-1 (A5222), based on the antibiotic Tiamulin (*from Pleurotus mutilus*)
- Myco-2 (A5233), based on the antibiotic Minocycline

**Features**
- Low costs
- 80% efficiency

**Form of delivery**
100X concentrated antibiotic solutions

**Shipping**
–20 °C

**Catalog numbers**
- A8360,0010 1 Set (2 x 10 ml)
- A8360,0020 1 Set (2 x 20 ml)
- A8360,0100 1 Set (2 x 100 ml)

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### Myco-3

**Application**
For the treatment of all mammalian cell lines including embryonic stem cells (ES cells).

**Components**
- Myco-3, based on the antibiotic Ciprofloxacin

**Features**
- Low costs
- 80% efficiency

**Form of delivery**
100X concentrated antibiotic solution

**Shipping**
–20 °C

**Catalog numbers**
- A8360,0010 10 ml
- A8360,0020 20 ml
- A8360,0100 100 ml

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### Myco-4

**Application**
1. For the treatment of very valuable cell cultures and week or very sensitive cell lines.
2. For elimination of mycoplasma species with known resistance against standard antibiotics.

**Components**
One kit is needed for a treatment. Each kit contains
- 1 vial of Starter Treatment solution (biophysical agent) and
- 3 vials of Main Treatment solution (antibiotics)

**Features**
- Lowest cytotoxicity
- Up to 100% efficiency
- No known resistance

**Form of delivery**
Sterile, ready-to-use solutions

**Shipping**
Cooled (2—8 °C)

**Catalog numbers**
- A8366,0002 2 Kits
- A8366,0005 5 Kits
There are two main reasons why mycoplasma contaminations are not banished from cell culture laboratories yet: First, **half of the researchers still do not test their cell cultures for mycoplasma** (Ryan 2008) and second, there is a tendency to rely on **antibiotics instead of good aseptic practices**.

Even though cell culture experts agree that general use of antibiotics can increase the severity of contamination problems, the routine use of antibiotics in cell culture laboratories is still prevalent. Particularly mycoplasma contamination rates are much higher in cell lines grown in antibiotic-containing medium than in antibiotic-free cultures (Barile 1973). If microorganisms, bacteria or fungi, are accidentally brought into antibiotic-free culture medium, they will replicate non-inhibited, soon leading to visible indicators of contamination: turbidity, filamentary structures, color changes due to pH alteration. In contrast, the presence of antibiotics will prevent the microbial growth – **maybe**. Unfortunately there is no absolute guarantee that the added antibiotics act against the introduced microorganisms (probably a mixture of different species), and sooner or later the user will face some kind of resistance phenomenon. If the introduced germ is fully resistant to the antibiotic, it will hopefully rapidly overgrow the culture and become visible within a short period of time. If the introduced microorganism only shows a partial resistance the situation is worse. Due to the latent static level of partly resistant contaminations, the risk of cross contaminations and usage of the affected culture in experiments or bio-production should not be underestimated. This worst case is very likely, if the invader belongs to the species of mycoplasma (e.g. brought into the culture through aerosol droplets from the mouth of the cell culturist), since **most common antibiotics used in cell culture do not act on mycoplasma**! Besides the beta-lactams being ineffective anyway, high resistance levels of mycoplasma against streptomycin (88%), kanamycin (73%), gentamycin (80%) and neomycin (86%) were determined (Lundin & Lincoln 1994).

Apart from Barile’s observation of strongly increased rates of mycoplasma contamination, morphological and functional changes are other disadvantages one has to take into account (Kuhlmann 1996), when using antibiotics on a routine basis. Anyhow, there exist useful applications for antibiotics in cell culture, e.g. within the first two weeks of primary culture. In order not to create new resistances due to inactivation of the antibiotic, the antibiotic-containing medium should be refreshed frequently.

As an alternative to classical cell culture antibiotics like penicillin-streptomycin, AppliChem provides a new product to prevent microbial growth in cell cultures: **CellCultureGuard**. This combination of selected antibiotics (one being a fluoroquinolone) offers a wide range of antimicrobial activity, making it our first choice cell culture reagent: **CellCultureGuard (A8906) is active against extra- and intra-cellular bacteria, mycoplasma, protozoa and fungi (yeast)**. Additionally, it is highly compatible with resistance markers and bears a low risk of resistance development.
References


For more information, please see the Mycoplasma Resource Centre at www.bionique.com


Please see our website www.applichem.com for products, protocols & more information.
### Test your culture – try AppliChem!

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<th>Description</th>
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<td><strong>Detection</strong></td>
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<td>A3744</td>
<td>PCR Mycoplasma Test</td>
<td>Ready-to-use (Taq Polymerase included!); contains a positive control DNA.</td>
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<td></td>
<td>Kit</td>
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<tr>
<td>A8994</td>
<td>PCR Mycoplasma Test</td>
<td>Highest sensitivity of &lt; 10 CFU/ml, according to Ph. Eur. (section 2.6.7). Besides a positive control DNA (non-infectious!) this kit provides an internal control DNA to visualize potential PCR inhibitions. The DNA Polymerase is not included, we recommend a Hotstart Taq Polymerase, e.g. SuperHot Taq DNA Polymerase (A5231).</td>
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<td>Kit II</td>
<td></td>
</tr>
<tr>
<td>A9019</td>
<td>qPCR Mycoplasma Test</td>
<td>Designed for qPCR applications. Provides a non-infectious positive control (FAM) and an internal control (ROX) to visualize potential PCR inhibitions. The DNA Polymerase is not included, we recommend a Hotstart Taq Polymerase, e.g. SuperHot Taq DNA Polymerase (A5231).</td>
</tr>
<tr>
<td></td>
<td>Kit</td>
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</tr>
<tr>
<td>A1001</td>
<td>DAPI</td>
<td>Excellent fluorescent dye for mycoplasma detection via DNA-staining followed by microscopy.</td>
</tr>
<tr>
<td><strong>Elimination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A8360</td>
<td>Myco-1 &amp; 2 Set</td>
<td>2-step treatment with tiamulin and minocycline.</td>
</tr>
<tr>
<td>A5240</td>
<td>Myco-3</td>
<td>Single-component treatment with ciprofloxacin.</td>
</tr>
<tr>
<td>A8366</td>
<td>Myco-4</td>
<td>2-step treatment with a mycoplasma-specific biophysical agent followed by an appropriate antibiotic combination.</td>
</tr>
<tr>
<td><strong>Prevention</strong></td>
<td></td>
<td></td>
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<tr>
<td>A5230</td>
<td>Incubator-Clean™</td>
<td>Non-toxic and biodegradable disinfectant for incubators and sterile benches; prevents contamination with and growth of fungi, bacteria (including mycoplasma) and viruses (including HIV and Hepatitis B). Fully compatible with common work surfaces (non-corrosive!).</td>
</tr>
<tr>
<td>A5219</td>
<td>Incuwater-Clean™</td>
<td>Non-toxic, non-volatile, and extremely effective disinfectant for CO₂ incubator water baths.</td>
</tr>
<tr>
<td>A9390</td>
<td>Aquabator-Clean™ (100X)</td>
<td>Disinfectant for prevention of microbial growth in common water baths.</td>
</tr>
<tr>
<td>A8906</td>
<td>CellCultureGuard</td>
<td>Combination of especially selected antibiotics to prevent microbial growth (extra- and intracellular bacteria, mycoplasma, protozoa and fungi) in cell cultures; provides high compatibility with resistance markers and low risk of resistance development.</td>
</tr>
</tbody>
</table>

Please note that AppliChem also provides • transfection reagents • growth factors and cytokines • vitamins • amino acids • antibiotics • antimycotics and other reagents for cell culture applications.
Vision
AppliChem was founded with the aim of supplying chemicals for chemical, biological, pharmaceutical and clinical research. It was also intended that AppliChem’s products should be available worldwide.

Experience
Our chemists have many years of in-depth experience and offer a sound partnership in helping to solve your problems in the lab. With you or for you – we want to develop new products. As well as flexibility, we assure you of strict confidentiality in all your projects.

Assortment
We prepare and provide you with chemicals and reagents including even those not listed in our current catalogs. When talking of “chemicals” in the widest sense of the word, we offer the service ’all products – one supplier’.

Quality
All AppliChem products are carefully controlled to guarantee that our customers continuously receive reliable highest quality. This is documented by certifications according to ISO 9001. Our products will fulfil your expectations and your individual, particular requirements are our business.

AppliChem is continuously gaining new customers, due to the exact and constant quality, as well as to the advantageous prices of our products and services. AppliChem is a reliable partner. Our quality control department provides detailed documentation on request.
There is another top address in Darmstadt:

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eMail service@de.applichem.com   Internet www.applichem.com