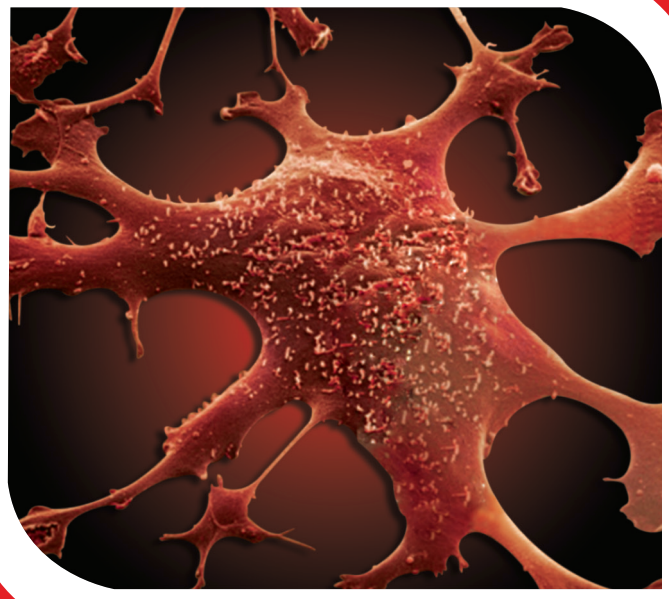
Mycoplasma Detection and Elimination Tools



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CellCulture
Enabling Breakthroughs

About Mycoplasma Contamination

Mycoplasma is one of the most prevalent and serious contaminant encountered in cell culture systems. Presently, contamination rates in established cell cultures have been reported between 15 - 35% with considerably higher occurrence (65 - 80%) cited in certain selected populations.

To overcome the problem of mycoplasma contamination in cell culture, HiMedia offers a complete solution by providing accurate, sensitive and reliable detection methods and robust and successful elimination methods.

Biology of Mycoplasma

- Smallest and simplest self-replicating prokaryotes belonging to genus Mollicutes
- Pleomorphic, lack cell wall and contain minimum organelles essential for cell growth and replication
- Circular, double stranded, genomic DNA with characteristically low G+C content
- Usually motile, aerobic organisms able to grow at temperature ranging from 20-45˚C
- Require sugars, arginine, cholesterol or related sterols for their growth
- Six species account for 95% cell culture contamination
- Majority of infecting mycoplasma are located extracellularly

Table 1: Most common Mycoplasma species causing cell culture contamination

Species	Frequency	Natural host
M. orale	20 - 40%	Human
M. hyorhinis	10 - 40%	Swine
M. arginini	20 - 30%	Bovine
M. fermentans	10 - 20%	Human
M. hominis	10 - 20%	Human
A. laidlawii	5 - 20%	Human

Sources of Mycoplasma Contamination

- Cross contamination with previously-mycoplasma infected cell line, specially new cell lines from external source
- Mycoplasma infected primary tissue used for the isolation of cells
- Use of media and reagents contaminated with mycoplasma as they can easily pass through membrane filters used for sterilization because of their small size
- From animal origin products like serum (bovine source) and trypsin (porcine source)
- Laboratory personnel with poor personal hygiene
- Poor laboratory conditions such as unclean lab apparatus, improperly maintained laminar air flow cabinets, etc

Effects of Mycoplasma Contamination on Cell Culture

- Alteration in growth characteristics, protein levels and synthesis of DNA and RNA
- Induction of chromosomal aberrations (numerical and structural)
- Induction of cell transformation
- Suppression of cytotoxic responses
- Cytotoxicity due to arginine depletion
- Decreased transcriptase activity
- Decreased virus production
- Interference with biochemical and biological assays
- Change in cell membrane composition (surface antigen and receptor expression)

In addition to these, mycoplasma contamination in hybridoma cultures impart serious implications such as decreased productivity, inhibition of cell fusion, interference in screening and conservation of hybridoma. This often leads to non-reproducible, non-reliable experimental results and unsafe biological products.

Why Mycoplasma Contamination Remains Undetected?

- Does not necessarily affect growth of cells and does not cause turbidity or change in colour of the medium
- Not visible under the microscope
- Resistance to routinely used antibiotics (such as penicillin) due to lack of cell wall
- Ability to pass through 0.45µm bacteriological membrane filters due to small size (0.3 - 0.8 µm) and pleomorphic cell membrane
- Ability to survive for long periods in aerosols and splashes

Mycoplasma Detection Tools

Mycoplasma contamination in cell culture can be detected by various methods such as microbial cultivation on agar and broth, DNA staining with fluorescent DNA stains, PCR, DNA-rRNA hybridization and ELISA.

Each of these methods have their own advantages and disadvantages. No particular method can be regarded as a "gold standard" for mycoplasma detection. Moreover, many of these methods rely on subjective reading and interpretation of results that require training, experience and consensus. Hence it is recommended to follow at least two detection procedures for routine screening of mycoplasma for cell culture.

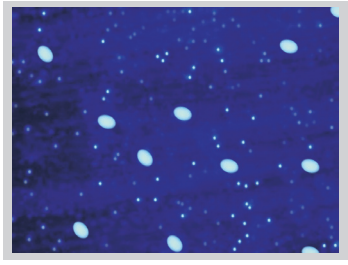
HiMedia provides ready-to-use, cost effective DNA staining kits and PCR-based kits for detection of Mycoplasma.

EZdetect™ DNA Stain Kits

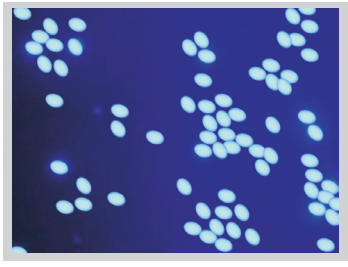
The **EZdetect™ DAPI and Hoechst Stain Kit for Mycoplasma Detection** are based on rapid uptake of the fluorescent stains DAPI and Hoechst by cellular DNA. DNA stains exhibit high permeability into cells; a property that facilitates quick uptake by the cells. They selectively bind minor grooves of the DNA. Their selectivity for DNA (over RNA) and high cell-permeability allow efficient staining of the nuclei. The excitation and emission spectra of Hoechst bound dsDNA are similar to DAPI bound dsDNA with excitation maxima around 350-360nm and emission maxima around 450-460nm. Both the stains can be excited either with a xenon mercury arc lamp or a UV laser and detected through a blue filter. Mycoplasma staining with these fluorescent stains appears as a fine particulate or filamentous staining over the cytoplasm at 100X magnification. Additionally, nuclei of cells are also brightly stained by this method and thereby act as endogenous positive control for the staining procedure.

Advantages

- Ideal for initial screening
- Easy to perform
- Rapid detection of Mycoplasma in just 45 minutes
- Binds to DNA with high sensitivity
- Detects bacteria, yeast and other prokaryotes



Mycoplasma-infected Jurkat cells with extra nuclear pinpoint fluorescence



Uninfected Jurkat Cells with nuclear fluorescence

Ordering Information

Product Name	Code	Packing
EZdetect™ DAPI Stain Kit	CCK007-100NO	Kit for 100 tests
EZdetect™ Hoechst Stain Kit	CCK008-100NO	Kit for 100 tests

Mycoplasma Detection Tools

EZdetect™ PCR Kits

Advantages

- Highly sensitive and rapid detection of mycoplasma
- Easy interpretation of results possible after gel electrophoresis
- High specificity of detection due to mycoplasma-specific primers
- Sensitivity - 1 cfu/ml of mycoplasma

Applications

- Suitable for cellular samples as well as non-cellular samples such as culture supernatant, media, sera & reagents
- Suitable in the field of vaccine production, biopharmaceuticals and cell therapy

EZdetect™ PCR kit for Mycoplasma Detection Based on 16s-23s rRNA spacer region

This kit is designed for detection of *Mycoplasma* based on amplification of intergenic transcribed spacer region (ITS) between 16S and 23S rRNA genomic DNA sequence. The ITS region is an important tool for the development of DNA-based classification because it shows a significant degree of variation in length and sequence from one species to another. This allows a clear distinction to be made between various strains of mycoplasma.

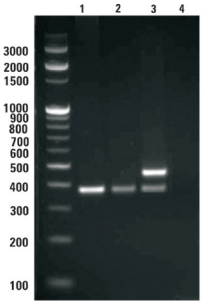
Species detected:

M. hyorhinis, M. fermentans, M. hominis, M. neurolyticum, M. orale, M. pirum, M. pulmonis, M. Salivarium, M. urealyticum

Amplicon size:

370 – 500 bp

Performance characteristics:



1. Positive control
2. Positive sample infected with one species
3. Positive sample infected with two species
4. Negative control

Gel run in 1X TAE buffer showing amplification of positive DNA (*M. Hominis* gDNA) and mycoplasma from infected cultures.

EZdetect™ PCR kit for Mycoplasma Detection Based on 16s rRNA sequence

This kit is designed for detection of *Mycoplasma* based on amplification of 16S rRNA genomic DNA sequence. This sequence is highly conserved between various mycoplasma species and can identify mycoplasma at both the genus & species level.

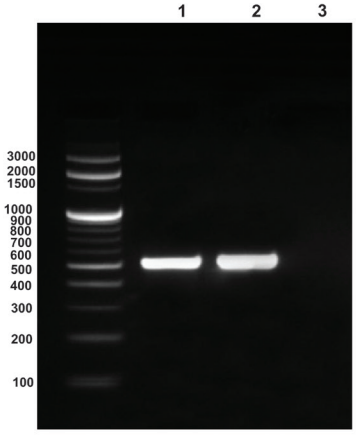
Species detected:

M. arginine, M. bovis, M. fermentans, M. hyorhinis, M. orale, A. laidawii, M. hominis

Amplicon size:

502 - 520bp

Performance characteristics:



1. Positive control
2. Positive sample infected with one species
3. Negative control

Gel run in 1X TAE buffer showing amplification of positive DNA (*M. Hominis* gDNA) and mycoplasma from infected cultures.

Ordering Information

Product Name	Code	Packing
EZdetect™ PCR kit for Mycoplasma Detection Based on 16s-23s rRNA spacer region	CCK009-25R	Kit for 25 reactions
EZdetect™ PCR kit for Mycoplasma Detection Based on 16s rRNA sequence	CCK022-25R	Kit for 25 reactions

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Mycoplasma Elimination Tools

Mycoplasma infected cultures should ideally be discarded after autoclaving. Attempts to eliminate mycoplasmas from contaminated cells should be considered as a last resort. Only in case where the infected cell line is very important and non-replacable, treatment for elimination can be considered. General procedures that have been used to eliminate mycoplasma from infected cultures include:

- Heat treatment
- Treatment with detergents and chemicals such as sodium polyanethol sulfonate and methyl glycine buffer
- Culture with specific anti-mycoplasma antisera
- Exposure to complement and chemotherapeutic treatments

Most of them have found to be ineffective in eliminating mycoplasma as they are too laborious, time consuming and often pose a risk of secondary infection to cell cultures. There is no such single method available that is 100% effective, however chemotherapeutic methods like antibiotic treatment have been reported to have a high degree of efficacy in eliminating mycoplasma from infected cultures.

HiMedia provides a convenient kit for successful elimination of Mycoplasma from infected cultures based on antibiotic treatment.

EZkill™ Mycoplasma Elimination Kit

EZkill™ Mycoplasma Elimination Kit is a ready-to-use antibiotic based kit for elimination of Mycoplasma from infected cultures. It involves treating the infected culture three times with alternating cycle of two antibiotic solutions.

Advantages

- Simple and inexpensive way of eliminating Mycoplasma.
- No marked cytotoxicity.
- No loss of cell characteristics
- Effective against : Mycoplasma, Acholeplasma and Spiroplasma

Kit contents

Code	Name	Quantity
CCK006 part A	EZkill™ Solution I, 100X	1 x 5ml
CCK006 part B	EZkill™ Solution II, 100X	1 x 5ml

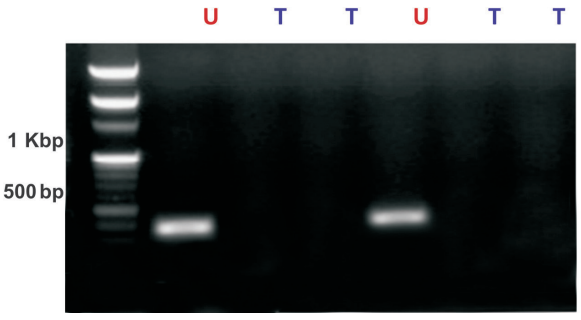
Part A: EZkill™ Solution I is ready to use 100X solution. It is a derivative of antibiotic pleuromutilin and mainly affects protein synthesis in Mycoplasma spp by binding 50S subunit of ribosome.

Part B: EZkill™ Solution II is ready to use 100X solution. It is a derivative of tetracycline and mainly affects protein synthesis in Mycoplasma spp by 30S ribosomal subunit.

Note: *Both the parts should be used one after the other. Do not use them together as it may cause cytotoxicity.*

It is recommended to use EZkill™ solutions only for elimination & not for prevention of contamination.

Performance Characteristics



U : Untreated control-CHO cells infected with Mycoplasma
T : CHO cells infected with Mycoplasma and treated with EZkill™

Ordering Information

Product Name	Code	Packing
EZkill™ Mycoplasma Elimination Kit	CCK006-1KT	1 Kit

Product Range

Ordering Information

Mycoplasma Detection Products

EZdetect™ DNA Stain Kits

Product Name	Code	Packing
EZdetect™ DAPI Stain Kit	CCK007-100NO	Kit for 100 tests
EZdetect™ Hoechst Stain Kit	CCK008-100NO	Kit for 100 tests

EZdetect™ PCR Kits

Product Name	Code	Packing
EZdetect™ PCR kit Based on 16s-23s rRNA spacer region	CCK009-25R	Kit for 25 reactions
EZdetect™ PCR kit Based on 16s rRNA sequence	CCK022-25R	Kit for 25 reactions

Mycoplasma Elimination Products

Product Name	Code	Packing
EZkill™ Mycoplasma Elimination Kit	CCK006-1KT	Sufficient for supplementing 500ml culture medium

Literature Code : TL264-2/Mycoplasma Detection and Elimination Tools/0317

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