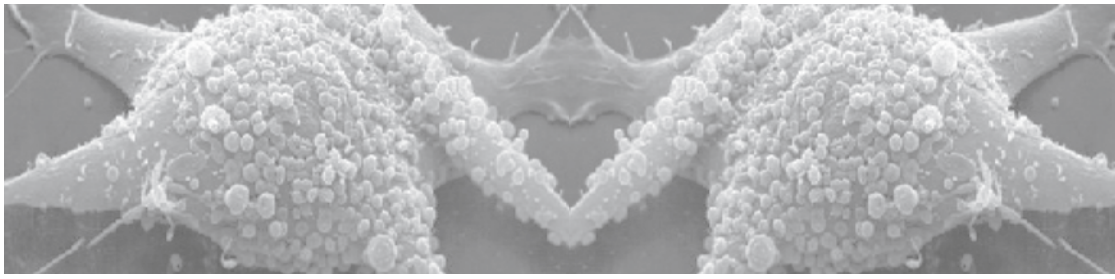
One Call

One Source

A World of Cell Biology Reagents



# MYCOPLASMAS:
## Detection and Decontamination

### Beware of Mycoplasmas: the most serious, widespread, and devastating culture contaminants.

Any handling of cell cultures poses the risk of contaminations with microorganisms. While bacterial and fungal infections of cultures are relatively easy to detect, to prevent and to treat, contamination with mycoplasmas represents a much bigger problem in term of incidence, detectability, prevention, eradication and effects.
More than 20 different mycoplasma species were isolated from cell cultures but the most frequently isolated, representing 80 - 90% of isolates are M. arginini, M. fermentans, M. hyorhinis, M. orale and Acholeplasma laidlawii.

### Why is the mycoplasma contamination rate so high ?

1. Mycoplasmas are the smallest self-replicating microorganism known (0.2 to 2µm in diameter). For this reason, mycoplasmas have the ability to to go through the filters used to sterilize cell culture media and sera, resulting in low levels of these organisms being accidentally introduced into cultures during routine feeding.
2. Mycoplasmas lack a rigid cell wall then, commonly used antibiotics that act on this structure like penicillin and streptomycin will not be effective.
3. Their small size and lack of a cell wall allow mycoplasmas to grow to very high densities in cell culture ($10^7$ to $10^9$ colony forming units/ml are common) often without any visible signs of contamination - no turbidity, pH changes or even cytopathic effects. Microscopic observation of live cell cultures cannot detect their presence.

### What are the consequences of mycoplasma contamination ?

1. Adverse effects on the cultures: alteration of growth characteristics, cell membrane composition, chromosomal structure etc…
2. Inaccurate or erroneous experimental results
3. Loss of valuable cell cultures

Therefore, regular testing for mycoplasmas is the only way to be sure of working with mycoplasma-free cell cultures leading to confidence in the quality and validity of data obtained.

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# Mycoplasma Stain Kit

- **Reliable**: Use of the Hoechst fluorescent stain method cited by the Tissue Culture Association (TCA procedure no.75361).
- **Versatile**: Detection of mycoplasma and bacteria, yeast, fungi.
- **Rapid**: The test takes less than 2 hours.
- **Complete**: Stain, diluent, mounting medium and controls are included in the kit.

The Mycoplasma Stain Kit uses a DNA fluorochrome staining method. A cell sheet between 50-80% confluent is fixed and stained with the Hoechst dye and examined under fluorescent microscopy.
The nature of the contaminants may be determined by its morphology, size and relationship to the cells. Mycoplasma will appear as very small bright extranuclear dots or rods. Other microbial contaminants will be larger.

## Results

Specimens should be observed by fluorescent microscopy (excitation wavelength : 360 nm and emission wavelength : 490 – 500 nm) at 400-1000X with oil immersion. Mycoplasma can be easily identified by comparing the results obtained with the positive and negative control slides.

## Negative culture

A negative culture will show only nuclear staining. Occasionally micronuclei or other nuclear fragments from dead or disrupted cells will appear as spherical bodies. They may be distinguished from mycoplasma by their large size and brighter fluorescence.

## Positive culture

A positive culture will show the cellular nuclei surrounded by small pinpoint dots of fluorescence. They will have a uniform size ranging from 0.1 – 0.3 µm in diameter and may be pleomorphic. The morphology may range from spherical bodies to filamentous-like forms.

## Kit contents

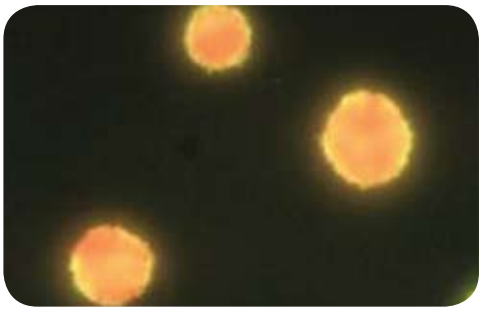
- Hoechst Stain 33258 - 1x10 ml
- 10X HBSS without phenol red and sodium bicarbonate - 1x10 ml
- Mounting Medium - 1x10 ml
- Positive/Negative fixed control slides – 5 slides

## Product Information

| Cat.# | Description | Size |
|---|---|---|
| 3030000 | Mycoplasma Stain Kit | 1 kit (100 tests) |

# ImmuMark™ Mycotest™ Kit

- **Rapid**: 30 min for the direct test / 60 min for the indirect test.
- **Versatile**: direct and indirect detection.
- **Sensitive**: specific staining of mycoplasma.
- **Complete**: control slides are included.



*Indirect immunofluorescent staining of M. bovis infected tumoral Ag-8 cell line (Magnification x 400)*

The ImmuMark™ MycoTest™ kit is an immunofluorescence test for the detection of mycoplasma in cell culture. It employs the monoclonal antibody CCM-2 specific for a common antigen produced by the most prevalent mycoplasma strains. The ImmuMark™ MycoTest™ offers two methods of detection:

- A **direct test** using the CCM-2 FITC-labelled monoclonal antibody in one step assay for fast screening of suspected positives.
- An **indirect test** using the labelled CCM-2 monoclonal antibody and a FITC-labelled secondary antibody for extremely sensitive mycoplasma detection.

Specimens are incubated for 20 minutes with either one or both conjugates and excess reagent is washed off with phosphate buffered saline (PBS). The mounted slides are viewed microscopically using fluorescent illumination (excitation wavelength 490 nm, emission wavelength 520 nm).

## Results

Yellow-green fluorescence is visible on the shape of infected cells or between cells which appear brightly red. In many cases mycoplasmas are crowded on a spot on the cell's surface.

Most common mycoplasma species recognized by the CCM-2 monoclonal antibody:

- Acholeplasma laidlawii
- Mycoplasma hyorhinis
- Mycoplasma arginini
- Mycoplasma orale
- Mycoplasma fermentans
- Mycoplasma salivarium

## Kit contents

- Monoclonal CCM-2 Antibody conjugated to FITC - 2ml
- Goat Anti-Mouse IgG conjugated to FITC (Secondary Antibody) - 2ml
- 2 control slides with a negative and a positive specimen
- Mounting Fluid - 2,5ml

## Product Information

| Cat.# | Description | Size |
|---|---|---|
| 3020000 | ImmuMark™ Mycotest™ Kit | 1 kit (50 tests) |

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# Mycoplasma Removal Agent - MRA

## Eliminate mycoplasma contamination without harming your cell lines

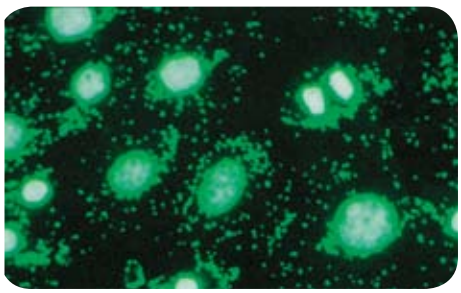
- **Strong anti-mycoplasma activity for a complete elimination of mycoplasma from contaminated cultures:**
  MRA is a derivative of the quinoline family of antibiotics and eliminates mycoplasma infection by inhibiting mycoplasma DNA gyrase, an essential enzyme for the microorganisms DNA replication.

- **Broad spectrum activity:**
  MRA acts in the cell culture for up to 7 days killing a wide variety of mycoplasma strains. It works at concentration as low as 0.5 µg/ ml.

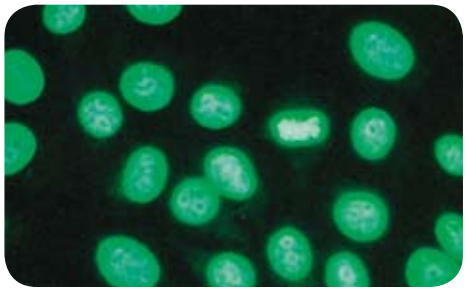
- **Effective at low concentration and proven low in cytotoxicity:**
  Cellular toxicity is rare when used at the recommended concentrations.That is why MRA can also be used to avoid mycoplasma contamination as a preventive measure

- **No recurrence of mycoplasma contamination:**
  Once treated with MRA, a cell culture will not be recontaminated with original mycoplasma while preventative doses of MRA are in use.

- **Very easy to use:**
  MRA can be used very conveniently. Simply add to cell cultures contaminated by mycoplasma and incubate for a week.



*Mycoplasma infected cell culture*



*MRA treated cell culture*

MRA is supplied as a ready-to-use solution and is stable at room temperature.

Each vial contains 5 ml of 4-oxo-quinoline-3-carboxylic acid derivative (concentration: 50µg/ml).

## Product Information

| Cat.# | Description | Size |
|---|---|---|
| 3050044 | Mycoplasma Removal Agent - MRA | 5 ml |

## References


1. O'Dea K.P., Pasvol G.: Optimal tumor necrosis factor induction by Plasmodium falciparum requires the highly localized release of parasite products. Infect. Immun., 71: 3155- 3164, (2003)
2. Rowe J.A., Scragg I.G., Kwiakowski D., Ferguson D.J.P., Carucci D.J., Newbold C.I. Implications of mycoplasma contamination in Plasmodium falciparum cultures and methods for its detection and eradication. Mol. Biochem. Parasitol., 92 : 177-180, (1998)
3. Peppel K., Jacobson A., Huang X., Murray J.P., Oppermann M., Freedman N.J. Overexpression of G protein-coupled receptor kinase-2 in smooth muscle cells attenuates mitogenic signaling via G protein-coupled and platelet-derived growth factor receptors Circulation, 102 : 793-799, (2000)
4. Trucco C., Javier Oliver F., De Murcia G., Menissier-de-Murcia J.: DNA repair defect in poly(ADP-ribose) polymerase-deficient cell lines. Nucleic Acid Res., 26 : 2644-2649, (1998).



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