Catalog Number: 3020000

Immu-Mark Myco-Test Kit

1. Intended Use

The Myco-Test is a direct immunofluorescence test for the detection of Mycoplasma species in cell culture.

2. Summary

Mycoplasma contamination of continuous cell lines is a major problem in biological research using cultured cells. It has been shown that mycoplasmas produce a variety of effects on cultured cells (e.g. changes in metabolism, immunologic or biochemical properties, growth, viability, etc.). Since mycoplasma infection in cell cultures normally is a chronic infection which may not be obvious by visual inspection or light microscopy, routine, periodic screening of cell cultures for mycoplasmas is important. A variety of tests to detect mycoplasmas in cell cultures have been developed (e.g. fluorochrome staining of DNA, monitoring toxic metabolites, culture method, etc.) but each method shows certain disadvantages. The major drawback of those methods is non-specificity or a very time-consuming procedure.

The Myco-Test contains a monoclonal antibody with specificity for a broad range of Mycoplasma species, including the species *Acholeplasma laidlawii, Mycoplasma bovis*, *Mycoplasma hominis, Mycoplasma hyorhinis*, *M. arginini*, *M. orale*, *M. fermentans*, *Mycoplasma pulmonis, Mycoplasma hyopneumoniae*and *M. salivarium*, which account for more than 96% of cell culture infections.[1,2] The test combines specificity due to monoclonal antibody reaction and very short assay time and provides - using the included fluorochrome-labelled secondary antibody - a very sensitive method for mycoplasma detection.

3. Principle of the Test

The Myco-Test provides two methods for mycoplasma detection: a direct test using a fluorochrome-labelled monoclonal antibody in a one step assay for fast screening of suspected positives and an indirect test using the labelled monoclonal antibody and a fluorochrome-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. The mounted slides are viewed microscopically using fluorescent illumination. If mycoplasmas are present, characteristic yellow-green fluorescence is seen on and between the counterstained red cells.

4. Reagents Provided

Each kit contains sufficient materials for testing 50 cell culture specimens. Materials are supplied ready to use.

4.1 Reagents

- One bottle containing 2 ml of monoclonal antibody, conjugated to FLUOS, diluted in a protein stabilized buffer solution with Evans blue as counterstain.
- One bottle containing 2 ml of Goat Anti-Mouse IgG, conjugated to FITC, diluted in a protein stabilized buffer solution with Evans blue as counterstain.

These reagents contain 0.1% (w/v) sodium azide. The reagents should be stored at 2-8^{o}C in the dark. The reagents should not be frozen.

4.2 Mounting Fluid

- One bottle containing 2.5 ml mounting fluid. The mounting fluid contains a photobleaching inhibitor in glycerol and should be stored at 2-8^{o}C.

4.3 Control Slides (MP Catalog No. 3020100)

- 2 control slides with a negative and a positive specimen on slide are included in the kit. They should be stored at 2-8^{o}C.

5. Precautions

The Myco-Test reagents contain 0.1% (w/v) sodium azide which is a poison. Sodium azide may react with copper and lead plumbing systems to form explosive metal azides. Always dispose of azide-containing materials by washing with large quantities of water.

Evans blue dye is present in the reagents. The dye is a possible carcinogen and contact with the skin should be avoided.

The mounting fluid may cause skin irritation and care should be taken when using it. Do not eat, drink, smoke, store or prepare foods within the designated working area. Do not pipette materials by mouth.

6. Collection and Preparation of Specimens

The collection and preparation of specimens is of fundamental importance for the detection of mycoplasmas in cell cultures. Do not subculture cells before applying immunofluorescence for at least 2 days and avoid media supplements which may interfere with mycoplasma growth (e.g. antibiotics like Gentamicin or Kanamycin). It is of great importance to include cells in the specimens. Pure culture supernatants should not be tested.

Adherent cells can be scraped off from their support or may be treated with trypsin. Trypsin has to be removed by washing after cells have been suspended.

Cells freshly thawed from liquid nitrogen storage can be used for immunofluorescence but should be washed once to eliminate the cryoprotectant.

As an inoculum of approximately 20,000 cells should be applied in one staining area, some cell cultures may have to be concentrated by centrifugation (approx. 500 x g for 5 to 10 minutes).

Preparation of Slides

Place approximately 20,000 cells in a volume of 20 to 30 ul into the 6 to 10 mm well area on a coated glass microscope slide and dry the sample at 50^{o}C for 45 minutes. Then fix for 60 seconds in -20^{o}C cold ethanol 70%. After fixation allow the slide to air dry at room temperature.

7. Test Procedure

7.1 One Step Staining

1. Add one drop of the FLUOS-labelled monoclonal antibody (Reagent 1) to the fixed cell preparation. Ensure that the reagent covers the entire well area. Incubate for 20 minutes at room temperature. Do not allow the reagent to dry on the specimen as it will cause non-specific staining.
2. Carefully rinse the slide with phosphate buffered saline and wash it twice for a total of 2 minutes in a bath of phosphate buffered saline.
3. Leave the slide to dry at room temperature.
4. Place one drop on mounting medium (Reagent 3) in the centre of each well and place a coverslip over the mounting fluid. Avoid trapping air bubbles.

7.2 Two Step Staining

1. Add one drop of the FLUOS-labelled monoclonal antibody (Reagent 1) to the fixed cell preparation. Ensure that the reagent covers the entire well area. Incubate for 20 minutes at room temperature. Do not allow the reagent to dry on the specimen as it will cause non-specific staining.
2. Wash the slide twice in phosphate buffered saline for a total of 2 minutes.
3. Add one drop of the Goat Anti-Mouse Fluorescein Conjugate (Reagent 2) to the fixed cell preparation. Ensure that the reagent covers the entire well area. Incubate for 20 minutes at room temperature. Do not allow the reagent to dry on the specimen as it will cause non-specific staining.

4. Wash the slide twice in phosphate buffered saline for a total of 2 minutes.
5. Leave the slide to dry at room temperature.
6. Place one drop of mounting medium (Reagent 3) in the center of each well and place a coverslip over the mounting fluid. Avoid trapping air bubbles.

NOTE: The two step staining method is not recommended for staining of hybridoma cells, as these cells secrete antibodies which are detected by the Goat Anti-Mouse Fluorescein Conjugate. In this case, background staining would be too high to detect Mycoplasma contamination. For screening of hybridoma cells for mycoplasmas, the one step staining method is recommended.

Please carefully follow this recommended procedure for specimen preparation. Modifications in the procedure will result in loss of sensitivity.

Do not use a cytospin for the preparation of specimens, as it would result in loss of sensitivity of the assay.

7.3 Controls

After unpacking a control slide, both wells are stained following the procedure described in section 7.1 or 7.2. The control slide contains a mycoplasma positive and a mycoplasma negative specimen.

7.4 Examination

Scan the well areas using a fluorescence microscope. Staining mycoplasma should be visible easily at x400 to x600 magnification. For best results specimens should be read immediately after staining.

8. Additional Reagents and Equipment Required

8.1 Reagents

- Ethanol 70% for fixation
- Phosphate buffered saline (pH 7.5) for washing stained specimens

8.2 Accessories and Equipment

- Fluorescence microscope with filter system for Fluorescein (maximum excitation wavelength 490 nm, mean emission wavelength 520 nm) and x400 to x600 magnification.
- Low speed centrifuge (optional) for concentration of cells
- Incubator (50^{o}C)
- Coated microscope slides (best are Teflon or PTFE-coated slides such as MP's Multitest slides, Catalog #'s 6040805, 6041805, 6041205 or 6041505)
- Wash bath
- Coverslips

9. Results

Yellow-green fluorescence is visible on the shape of infected cells or between cells which appear brightly red. Mycoplasma must not be present on all cells of the specimen. In many cases mycoplasmas are crowded on a spot on the cell's surface. Depending on the mycoplasma species present in the specimen, the shape of the stained bacteria may vary from very small, coccoid bodies with bright fluorescence to short filaments which may be stained more diffusely.

10. Sensitivity and Specificity

In an independent study 42 cell lines of different origin were tested for Mycoplasma infection comparing the Myco-Test (single step procedure) with the microbiological (culture) method as "golden standard"[3]. The results were as follows:

Mycoplasma Infection

	positive	negative
Culture	29	13

Myco-Test 30* 12

* The single false positive result was obtained with the only insect cell line in this study. It has to be evaluated, whether insect cell lines should be excluded as specimens for the Myco-Test.

11. Bibliography

1. Blazek, R, Schmitt, K., Krafft, U., Hadding, U.: "Fast and simple procedure for the detection of cell culture mycoplasmas using a single monoclonal antibody." *J. of Immunological Methods*, **v. 131** (1990) 203-212.

2. Kamla, V., Henrich, B., Hadding, U.: "Species differentiation of mycoplasmas by EF-Tu specific monoclonal anitbodies." *J. of Immunological Methods*, **v. 147** (1992) 73-81.

3. Hopert, et. al. "Specificity and sensitivity of Polymerase Chain Reaction (PCR) in comparison with other methods for detection of mycoplasma contamination in cell lines." *Journal of Immunological Methods*, 164 (1993) 91-100.