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Mycoplasma Staining Kit

09/20

(Catalog # K1482-2, -10; 2 or 10 ml; Store at 4°C)

I. Introduction:

BioVision's Mycoplasma Staining Kit provides an ultrasensitive, rapid and simple fluorescence based microscopic assay for the visual identification of mycoplasma infections in laboratory cell cultures. Mycoplasma infections are relatively common in laboratory cell cultures. It has been estimated that between 5% and 35% of all cell cultures are infected. Mycoplasmas have been shown to alter the growth rate of cells in culture, induce chromosomal aberrations, influence amino acid and nucleic acid metabolism and cause membrane aberrations. **BioVision's Mycoplasma Staining Kit** contains a MycoFluor Reagent and Antifade Mounting Medium. The MycoFluor reagent is added directly to the culture medium, with or without cells, and the stained sample is then examined under a fluorescence microscope with an excitation and emission maxima of 350-360 nm and 450-460 nm respectively. Mycoplasma staining with MycoFluor reagent appears as fine particulate or filamentous staining in the cytoplasm at 100X magnification. Nuclei of the cells are also brightly stained by this method and thereby act as endogenous positive control for the staining procedure.

II. Application:

- Detection of Mycoplasma in both suspension and adherent cell cultures

III. Key Features:

- **Ultrasensitive**
- **Rapid**, results in 60 min or less
- Visual identification
- **Ready-to-use**

IV. Sample Types:

- Adherent and suspension cell cultures

V. Kit Contents:

Components	K1482-2 (2 ml)	K1482-10 (10 ml)	Part Number
MycoFluor Reagent	2 ml	10 ml	K1482-XX-1
Antifade Mounting Medium	2 ml	10 ml	K1482-XX-2

VI. User Supplied Reagents and Equipment:

- Sterile tissue culture grade water
- Sterile PBS buffer
- Carnoy's fixative (3:1 Methanol: Glacial acetic acid)
- Methanol
- Glacial acetic acid
- Centrifuge tubes
- Glass slides
- Microscope coverslips
- Fluorescence Microscope

VII. Shipping and Storage Conditions:

The kit should be stored at 4°C and in the dark. The kit is light sensitive. The kit reagents are stable for 6 months if stored as recommended.

VIII. Reagent Preparation and Storage Conditions:

Prepare Carnoy's fixative freshly using methanol and glacial acetic acid in the ratio 3:1 respectively.

IX. Staining Protocol:

The kit can detect Mycoplasma in both suspension (non-adherent) and adherent cell cultures.

A. Staining of suspension cells

1. Aseptically aspirate the culture medium containing suspension cells from the culture vessel and transfer it to a sterile centrifuge tube.
2. Centrifuge the tube at 1000 rpm for 10 min at room temperature (RT).
3. Discard the supernatant and resuspend the pellet in 500 µl of medium.
4. Add 1 ml of freshly prepared Carnoy's fixative and mix well.
5. Centrifuge at 1000 rpm for 10 min at RT.
6. Discard the supernatant and resuspend the pellet in 500 µl of sterile PBS buffer.
7. Apply one drop of MycoFluor reagent and mix well.
8. Allow it to stand for 15-20 min at RT in the dark.
9. Add one drop of the suspension on a clean, grease-free slide and make a thin smear. Allow it to air dry.
10. Apply one drop of anti-fade mounting medium on the smear and put a coverslip on it.
11. Observe the slide under a fluorescence microscope with an excitation and emission maxima of 350-360 nm and 450-460 nm respectively. Observe the slides using objectives of magnification 40X or 100X. The slides can also be observed using a 100X oil immersion objectives.

B. Staining of adherent cells

Cells should be grown at 50-80% confluence before use. Cells could be grown on slides or chamber slides or on coverslips in Petri dish or 6-well tissue culture plates.

1. Aspirate the medium from the cell culture vessel

2. Add sufficient volume of freshly prepared Carnoy's fixative to cover the monolayer completely.
3. Allow it to stand for 10 min at RT.
4. Remove the fixative.
5. Add 1 ml of sterile PBS buffer. Then apply one drop of MycoFluor reagent and mix well.
6. Allow it to stand for 15-20 min at RT in the dark.
7. Remove the left over stain solution and allow it to dry.
8. Mount the slide/coverslip as follows:
    a. Slide: Apply one drop of anti-fade mounting medium on the upper cell sheet surface of the slide and cover with a coverslip.
    b. Coverslip: Apply one drop of anti-fade mounting medium to a glass slide. Put the coverslip on the mounting medium with cells side down.
9. Observe the slide under a fluorescence microscope with an excitation and emission maxima of 350-360 nm and 450-460 nm respectively. Observe the slides using objectives of magnification 40X or 100X. The slides can also be observed using a 100X oil immersion objectives.

X. Interpretation of the results:

1. Negative result: If the culture is negative for Mycoplasma, then the sample will show only nuclear fluorescence. Occasionally micronuclei or nuclear fragments from dead and disrupted cells will appear as spherical bodies. Their large size and brighter fluorescence will distinguish them from Mycoplasma.
2. Positive Result: If the culture is positive for Mycoplasma, then the sample will show extra-nuclear fluorescence along with the nuclear fluorescence. Mycoplasma can be identified by small pin point dots of fluorescence, either aggregated in clusters or scattered uniformly in the cytoplasm and sometimes in the intercellular spaces.
3. Bacteria, yeast and other prokaryotes show typical size, morphology and growth characteristics (i.e. chains, budding, mycelia, etc.).
4. It is necessary to completely scan the specimen slide or test slide before interpreting the results because all the cells may not be infected with Mycoplasma. Incomplete scanning may result in false negative results.
5. If there is any doubt regarding the interpretation of the fluorescence, the staining should be repeated after generating a further subculture of the test cells in the absence of antibiotics.
6. Further confirmation of the Mycoplasma infection can be done using other assays such as PCR, ELISA or direct growth on agar or in broth.

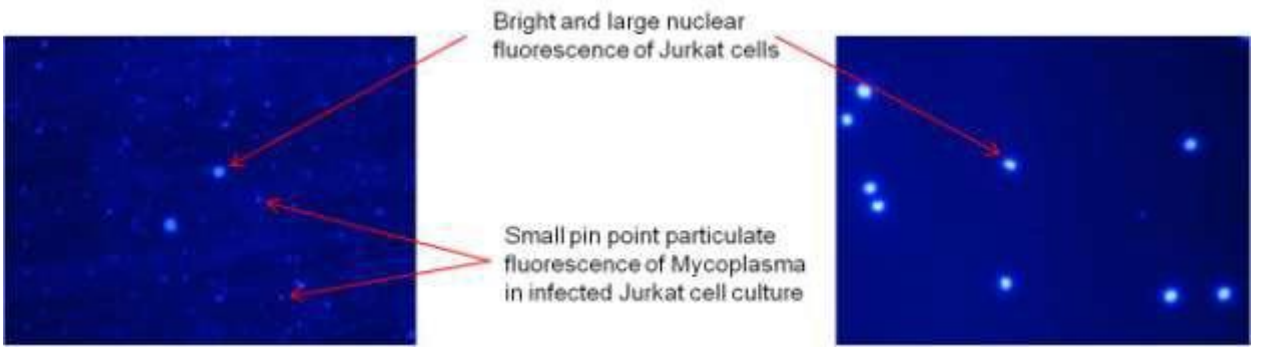

Figure 1 showsJurkat cells stained using Mycoplasma Staining Kit

XI. Related Products:

BioVision Product Name	Cat. No.	Sizes
Mycoplasma PCR Detection Kit	K1476	**100 Rxns**
Mycoplasma DNA Kit	K1416	50, 250 Preps
Mycoplasma Pneumoniae IgG ELISA Kit	E4664	96 Assays
Mycoplasma Pneumoniae IgM ELISA Kit	E4665	96 Assays
Mycoplasma Arginine Deiminase (ADI), Recombinant Protein	P1278	5 µg, 20 µg