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Mycoplasma Stain Kit

Cat. #: C0045 Size: 10 ml

Introduction

Mycoplasma Stain Kit provides an ultrasensitive, rapid and simple fluorescence microscopic assay for the visual identification of mycoplasma infection in laboratory cell cultures. In order to detect mycoplasma, the fluorescent MycoFluor reagent is added directly to the culture medium, with or without cells, and the stained sample is then examined under a fluorescence microscope. The excitation and emission spectra of MycoFluor reagent bound to dsDNA are similar to dsDNA bound to DAPI, with excitation maxima around 350-360nm and emission maxima around 450-460nm. MycoFluor reagent can be excited either with a xenon mercury arc lamp or a UV laser and is detected through a blue filter.

Mycoplasma staining with MycoFluor reagent appears as a fine particulate or filamentous staining over 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.

Package Information

Components	M0045
MycoFluor Reagent	10 ml
Antifade Mounting Medium	10 ml

Storage

Store at 2-8°C and protect from light.

Protocols

The kit has been designed to detect Mycoplasma in both suspension (non-adherent) and adherent cell cultures.

1. Staining of suspension cells

1.1 Aseptically aspirate the culture medium containing suspension cells from the culture vessel and transfer it to a sterile centrifuge tube.

1.2 Centrifuge the tube at 1000rpm for 10 minutes at room temperature.

1.3 Discard the supernatant and resuspend the pellet in 500µl of medium.

1.4 Add 1ml of freshly prepared Carnoy's fixative and mix well.

1.5 Centrifuge at 1000rpm for 10 minutes at room temperature.

1.6 Discard the supernatant and resuspend the pellet in 500µl of sterile PBS buffer.

1.7 Apply one drop of MycoFluor reagent and mix well.

1.8 Allow it to stand for 15-20 minutes at room temperature, in dark.

1.9 Add one drop of the suspension on clean, greasefree slide and make a thin smear. Allow it to air dry.

1.10 Apply one drop of anti-fade mounting medium (Component B) on the smear and put a coverslip on it.

1.11 Observe the slide under fluorescence microscope.

2. Staining of adherent cells

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

2.1. Aspirate the medium from culture vessel

2.2. Add sufficient volume of freshly prepared Carnoy's fixative to cover the monolayer completely.

2.3. Allow it to stand for 10 minutes at room temperature.

2.4. Remove the fixative.

2.5. Add 1ml of sterile PBS buffer, then apply one drop of MycoFluor reagent and mix well.

2.6. Allow it to stand for 15-20 minutes at room temperature in dark.

2.7. Remove the left over stain solution and allow it to dry.

2.8. Mount the slide/coverslip as follows:
a. Slide - Apply one drop of anti-fade mounting medium on the upper cell sheetsurface 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 cell side down.

2.9. Observe the slide under fluorescence microscope.

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