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MycoFluor Mycoplasma Detection

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We use this protocol and it's working.

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Abstract

MycoFluor™ Mycoplasma Detection produces a fast and simple fluorescence microscopic assay that identifies mycoplasma infection in cell cultures. In order to detect mycoplasma, the fluorescent MycoFluor™ reagent is added to the culture medium, with or without cells, and the sample becomes stained and examined under a fluorescence microscope.

Materials

Reagents Needed:

1. MycoFluor Mycoplasma Detection Kit (M-7006)
2. Microcentrifuge tubes
3. Microscope slides
4. Clear coverslips

Troubleshooting

Protocol Testing of Culture Media

1 Take about 4mL of cell medium directly form the culture dish in which the cells have been growing centrifuge the sample at 1300 x g for 10min to pellet any cells and debris

2 Carefully transfer 1mL of supernatant into labeled microcentrifuge tubes.

3 Centrifuge the microcentrifuge tubes at 12,500 x g for 15 minutes

4 Carefully remove and discard 0.5mL of supernatant, leaving behind 0.5mL of medium in the tube. Resuspend any pellet that may have formed using this 0.5mL of medium.

5 Add 26µL of 20X concentrated MycoFluor reagent to 0.5mL of the medium.

6 Pipet 10µL of the stained medium onto a clean microscope slide and cover with a clean coverslip.

7 Seal the slide using quick dry clear nail polish topcoat by covering all sides of the coverslip.

8 Image the slide using fluorescent microscope.

Control Slides with Mycoplasma MORFS

9 Generating positive controls for the testing of culture media

9.1 a. Pipet 5µL of the mycoplasma MORFS stock suspension on a clean, labeled microscope slide

9.2 b. Add 5µL of stained medium to the slide

9.3 c. Cover with clear coverslip and seal using quick dry topcoat nail polish.

10 Image the positive controls and compare to the samples that have not been spiked with MORFS

Microscopy

11 Prepare the microscope with a near ultraviolet fluorescence filter (Excitation at 365nm and either bandpass 450nm ± 30nm or longpass >400nm emission filter)

12 For optimum result, a 100X oil immersion objective is suggested but a 60X oil immersion objective will also work

13 First look at the control slide spiked with MORFS to get an idea what it should look like. Then examine the test slide for extranuclear blue fluorescence.