SIGMA®

ProductInformation

Mycoplasma Detection

In situ DNA fluorescence is a very efficient method of screening for mycoplasma contamination in cell cultures. Bisbenzimide (Hoechst 33258) and DAPI (4', 6-Diamidino-2-phenylindole) are DNA fluorochromes which bind specifically to the Adenine-Thymidine (A-T) regions of DNA. Cultures contaminated with mycoplasma will have small, uniformly shaped fluorescent bodies evident in the extranuclear and intracellular spaces. Nuclei of cultured cells will also fluoresce.

Artifacts may fluoresce and interfere with interpretation. They will appear larger in size than mycoplasma and irregular in shape. Using healthy, log-phase indicator cells and test cells will reduce interference caused by artifacts.

MATERIALS

Indicator cells, Vero (ATCC™ CCL 81) or 3T6-Swiss albino (ATCC™ CCL 96)
Leighton tubes or glass cover slips/culture dishes
Cell culture medium (growth medium)
Methanol
Glacial acetic acid
Bisbenzimide (Product No. B 1155) or DAPI (Product No. D 8417)
Mounting Solution McIlvaine's Buffer: Glycerol [1:1] (Product No. M 7534)
Fluorescent microscope **(see Procedure: Examining cultures)**

PROCEDURE

Culturing samples and indicator cells
1) Seed indicator cells at low density in a Leighton tube or on a glass coverslip in a culture dish containing tissue culture medium. Incubate for 24 hours at the conditions appropriate for the culture medium (typically 37°C at 5% or 2% CO_2). Prepare enough cultures to inoculate with control and test samples.
2) To separate indicator cell cultures, add 0.1 ml of test samples. Negative control: Indicator cell cultures inoculated with 0.1 ml of culture medium. Positive control: If a positive control is desired, infect a culture of the indicator cells with 0.1 ml of a viable culture of mycoplasma species.
3) Allow all cultures to incubate for an additional 4 days.
NOTE: It is important to stain and examine cultures before they reach confluency. Adjust incubation time and inoculum density according to the growth characteristics of the test and indicator cells.

Fixing Cells
1) Prepare Carnoy's fixative fresh on the day of use. Solution consists of 3 parts methanol to 1 part glacial acetic acid. Prepare enough solution to fix all cultures. Approximately 15 ml of fixative is required per culture.
2) Without decanting growth medium, add approximately 5 ml of Carnoy's fixative to each culture and allow to stand 2 minutes.
3) Decant and add 5 ml of fixative to the cultures and allow to stand 5 minutes.
4) Decant fixative, add 5 ml of fresh fixative, and allow to stand 5 minutes.
5) Finally, decant fixative and allow growth surface to air dry approximately 5 minutes.

Staining and Mounting Cells
1) Prepare working concentration of fluorochrome stain (Bisbenzimide) by dissolving 0.25 - 0.5 µg/ml of distilled water. Concentration of stock solution should be

50 µg/ml and stored in the dark. Stock solution should be sterile and discarded if performance deteriorates.

Note: DAPI may be substituted for bisbenzimide. Solubilize DAPI water at 0.1 mg/ml. Stain cells for 15-30 minutes.

2) Completely immerse the growth surface in the stain solution and allow to stand for 30 minutes.

3) Rinse twice with distilled water.

4) Mount growth surface, cell side down, with a drop of mounting solution on a microscope slide. Slides may be preserved by sealing the edges of the cover slip and slide with clear nail polish. Slides should be protected from light and heat. These will last several weeks without quenching if properly stored.

Sigma offers a Mycoplasma Stain Kit (MYC-1) which contains all the reagents necessary to perform the Hoechst staining procedure described. The kit also contains positive and negative control slides for comparison with test slides.

Examining cultures

A fluorescent microscope capable of epifluorescence is needed for visualizing the stain preparations. A typical system includes fluorescent microscope with a 53/44 barrier filter and a BG-3 exciter filter. A total magnification of 500X (40X; 12.5) is usually sufficient to visualize mycoplasma but higher magnification may be used.

REFERENCES

1. Chen, T.R. (1977). Exp. Cell Res. 104:255-262.
2. Hay, R.J. et al. (1989). Nature 339:487-488.
3. McGarrity, G.J. et al. (1983). In: Methods in Mycoplasmology Vol. 2. Tully and Razin (eds). Academic Press, Inc. New York, NY pp. 487-488.

ATCC® is the registered trademark of the American Type Culture Collection