Mycoplasma Screening of Cultured Cells by Fluorescence Microscopy

Prepare cell samples

Make sure cells have been passages at least 2 times after a thaw so mycoplasma have recovery time from their time in cold storage!!
1. With sterile tweezers or Pasteur pipet attached to vacuum flask place one 22x22mm or 25mm round sterile glass coverslip into well of sterile 6well plate (# wells = # cell lines tested) or 35mm dish.
2. Add 3 ml of (cell line specific) complete medium without antibiotics (**important because they may inhibit mycoplasma and produce a false negative**) slowly to the well (touch first drop of medium to the edge of the coverslip until capillary action carries the liquid under the coverslip and completely covers the underside, which holds the coverslip to the dish and disallows cell growth on the underside).
3. Add 2-4 drops of cell suspension (approximately 10^5cells) to the medium in the well. Rock gently.
(Note: For testing non-adherent cells, a negative indicator line must be used. The line to be tested must be coplated with the indicator line. Mycoplasma will transfer to the indicator line within 2 days and will be visualized.)
4. Incubate 2-3 days at appropriate temperature for cell line until they are approximately 70% confluent (mycoplasma live on the outer membrane, and thus are easier to visualize when cells are subconfluent).

To Fix
1. Add 3 ml methanol gently to the side of the dish (do not remove growth medium).
2. Leave for 3 min at room temperature.
3. Aspirate methanol/medium solution.
4. Add 3 ml methanol for 15 min at room temperature.
5. Aspirate methanol and add 3 ml PBS or any isotonic saline solution (if sample is to be stored for any length of time, add PBS and Azide and store at 4oC for up to a month.

To Visualize (to be done by Cell Culture Facility Staff)
1. Place a drop of stain (Hoechst, preferably, or Dapi) on a labeled slide.
2. With an 18 gauge needle, lift up edge of coverslip from dish. Remove coverslip and dry underside (cell-free surface) with a tissue. Tilt and pat off excess liquid at edge.
3. Place the coverslip (cell-side down) onto the stain. With a tissue, dry any excess stain from the edge of the coverslip. Approximately six slides may be prepared at one time. 4. Place oil on coverslip and examine under the fluorescence microscope using the 63X or 100X objective.

Hoescht Stain: 2-(2-(4-hydroxylphenyl)-6-Benzimidazolyl)-6-(1-methyl-piperazyl)-benzimiadazole-HCl; #33258 Hoescht fluorescent stain, Polysciences, (215) 343-6484, Cat. #9460. 1000X stock solution = 5mg/ml, working solution 1X 5µg/mL in PBS.

Dapi Stain: 4',6-diamidino-2-phenylindole; #18860 Dapi fluorochrome powder, Accurate Chemical & Scientific Corp. (516) 433-4900. 1X solution = 5µg/mL in buffer (may be better at 0.5µg/mL).