DAPI MYCOPLASMA ASSAY

IMPORTANT NOTE

This is a simple visual assay for mycoplasma contamination that should be performed before freezing down any cell lines or if contamination is suspected. Since preparing the treated glass cover slips takes about a day they should be prepared in bulk beforehand (once treated they can be stored in a sterile tissue culture dish at room temperature indefinitely).

EQUIPMENT REQUIRED

Hot plate
Glass beaker
Two (or more) 100 mm sterile tissue culture dishes
Whatman/Blotting paper
Tweezers
Six well dish(es)
Glass cover slips (Fisherbrand #12-540B 22 x 22 mm) and microscope slides

REAGENTS REQUIRED

A. Treating Cover slips

- 1M HCl
- dH_2O
- 100% Ethanol
- Poly-D-Lysine solution (50 µg/mL in dH_2O)

B. DAPI Assay

- DAPI working solution:
  - Dilute 1 µL of DAPI stock (1000X or 5 mg/mL in dH_2O) in 10 mL 1X PBS.
- Methanol (pre-cooled to -20 °C)
- 1X PBS
- Anti-fade aqueous mounting media (or glycerol in a pinch)

A. TREATING COVER SLIPS

1 In the fume hood place cover slips to be treated in a glass beaker and cover with 1 M HCl.

2 Loosely cover the beaker to prevent excess evaporation (don't use tinfoil!) and heat at 50 °C using a hot plate for 4 - 6 hours.

3 Turn off the hot plate and allow the beaker to return to room temperature.

4 Wash 3X in dH_2O to remove any HCl residue.

5 Rinse the cover slips in 100% ethanol and let them dry between Whatman/blotting paper.

6 Place cover slips in a tissue culture dish and incubate them with Poly-D-Lysine solution on a rocker for one hour.

7 Wash 5X in dH_2O to remove any free Poly-D-Lysine.

8 Rinse cover slips in 100% ethanol and dry in the tissue culture hood propped up on the edge of a tissue culture dish (sterility is important here!).

9 Store the dried cover slips in a sterile tissue culture dish until use.

B. DAPI ASSAY

1 Using sterile tweezers (wipe them down with 70 % ethanol) place a single treated cover slip in each well of a six well dish.

2 Plate the cells to be tested at 100 000 cells per well.

3 Incubate overnight allowing the cells to adhere to the glass cover slips.

4 In the morning aspirate off all traces of media.

5 Fix and permeabilize the cells with 1 mL of -20 °C methanol per well, incubate the plate at -20 °C for 10 minutes.

6 Aspirate off the methanol and stain each well with 1 mL of DAPI working solution at room temperature for 10 minutes – remember to keep the plate protected from light!

7 Wash once with 1X PBS to remove any excess DAPI.

8 Carefully remove the cover slip from the six well dish using tweezers.

9 Mount with any aqueous mounting media (glycerol works fine but wont offer any protection against bleaching)

10 Visualize at 200X for mycoplasma contamination.

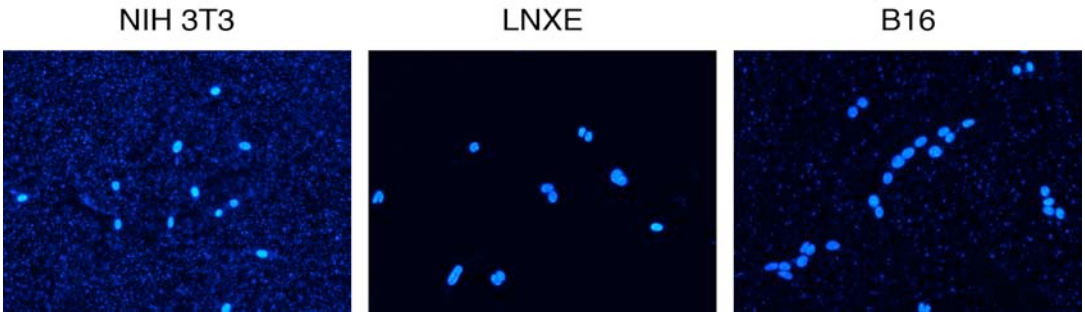

Figure 1. Example slides showing mycoplasma contaminated (NIH 3T3 and B16) vs. clean cells (LNXE). Mycoplasma appear as granulation or small DAPI positive spots both outside and inside of the cells