

Protocol Mycoplasma Detection Test

Mycoplasma refers to a genus of bacteria that lack a cell wall. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other beta-lactam antibiotics that target cell wall synthesis. Mycoplasmal cell culture contamination occurs due to contamination from individuals or contaminated cell culture medium ingredients. Mycoplasmas may induce cellular changes, including chromosome aberrations, changes in metabolism and cell growth, changes in responses to stimulus and then difficulties to repeat your experiments. Severe Mycoplasma infections may destroy a cell line.

An easy way to test and confirm the presence of mycoplasma in your cell culture is to do a DAPI staining on your cells.

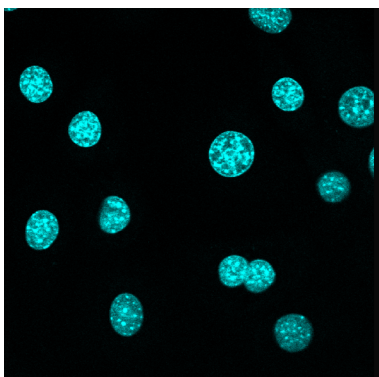
Material

- Cells are cultured on glass bottom dish (1 dish/ cell type to test)
- Cell confluence does not represent an issue, since you want to check the presence or the absence of mycoplasma contaminants in the cytoplasm.
- 4% PFA solution
- 1X PBS
- DAPI solution (1/5000 dilution from stock solution 1mg/ml) (Thermo scientific, product # 62248)

Protocol

1. Remove cell culture medium and incubate cells for 15 minutes with 4% PFA at room temperature.
2. Wash the cells twice with 1X PBS.
3. Incubate cells with DAPI solution for a minute.
4. Wash the cells 2-3 times with 1X PBS.
5. Keep the cells in PBS.
6. Observation can be done with classic epifluorescent microscope, but better results and better definition will be obtained using the Olympus Fv10i confocal microscope.
7. If contamination is detected, please trash cells and thaw a new batch of cells.

Mycoplasma negative



Mycoplasma positive

