

Filipin Fluorescence Staining of Free Cholesterol in Cultured Cells

Filipin Complex: Sigma (F-9765)
Stock: 25 mg/ml in DMSO
Working Solution: 0.05 mg/ml in PBS/10% FBS

Note: Protect filipin solutions from light!!

Procedure:

- 1) Rinse cells 3X with PBS.
- 2) Fix with 3% paraformaldehyde (fresh) for 1 h at room temperature.
- 3) Rinse cells 3X with PBS.
- 4) Incubate with 1 ml of 1.5 mg glycine/ml PBS for 10 min at room temperature to quench the paraformaldehyde.
- 5) Stain cells with 1 ml of filipin working solution for 2 h at room temperature.
- 6) Rinse cells 3X with PBS.
- 7) View cells in PBS by fluorescence microscopy using a UV filter set (340-380 nm excitation, 40 nm dichroic, 430-nm long pass filter). Note that filipin fluorescence photobleaches very rapidly.