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—this can be avoided by using a neutral density filter that lets through only 1, 5, or 10% of light.