Cholesterol Depletion of Hela Cells using Methyl-Beta-Cyclodextrin in LPDS

Reagents:

methyl-B-cylcodextrin: 13.1165 mg/mL; directly dissolve powder to medium, then filtrate.

Lipoprotein Deficient Serum (fetal calf): Mix LPDS in plain DMEM (1:9) to make 10% LPDS-DMEM.

LPDS should be heat-inactivated at 56 degrees celcius for 30 min.

1. Seed 3X10^5 hela cells per each well to coverslips in 10% FBS/l-glut/penn strep DMEM and incubate O/N.
2. In the morning prepare 10mM methyl-β-cyclodextrin/10% LPDS-DMEM.
3. Wash Hela cells with 2 mL/well of 37 degrees celcius pre-warmed plain DMEM
4. Add 1 mL of 10 mM methyl-β-cyclodextrin/10% LPDS-DMEM to each well and incubate for 1 hr at 37 degrees celcius. You can try going to 2 hrs but this may affect cell viability too much.
5. Wash cells with pre-warmed PBS.
6. Add 1 mL of 10% LPDS-DMEM and incubate at 37 degrees celcius for 1 hr.
7. Wash coverslips in PBS.
8. Fix cells with 2% Formaldehyde/PBS for 25 min at RT.

Immunostaining

1. To make diluent add 50 ug/mL of filipin/ethanol to 0.2% saponin/0.1% BSA/0.05% NaN_3.
2. Place a sheet of parafilm in a 15 cm dish and aliquot 20 uL of primary antibody/ saponin-filipin solution for each sample.
3. Remove coverslip from dish and blot using a kim wipe and place coverslip onto drop of primary antibody/saponin-filipin. Incubate 1 hr at room temperature or 30 min at 37 degrees celcius. Place a wet kim wipe in dish when incubating to maintain humidity.
4. Slip each coverslip into PBS and rinse for 10 min in a washer.
5. For secondary antibody use the same filipin/0.2% saponin/0.1% BSA/0.05% NaN_3 diluent. Again blot each coverslip using a kim wipe and then place onto drop containing secondary antibody/saponin-filipin solution. Incubate at either 1 hr at RT or 30 min at 37 degrees celcius. Because secondary antibodies are usually light sensitive keep them covered from light by wrapping dish in foil.
6. Rinse coverslips for 10 min.

8. Mont each coverslip onto a drop of mounting solution onto a slide. Filipin staining has a tendency to quench quickly so you might want to use antifade mounting solution instead of Flouromount G.