II. Immunofluorescence – Saponin

Saponin is a mild detergent that removes cholesterol from plasma membranes.
At low concentrations, internal membranes will remain intact.
Updated 12/22/93, Rewritten 9/28/05 – Wandinger-Ness lab

Reagents

0.5% Saponin/80mM Pipes, pH 6.8; 1 wash total, 2ml/well
   5mM EGTA
   1mM MgCl₂

0.05% Saponin/PBS(-); 9 washes total, 2ml/well

0.05% Saponin/PBS(-) (for dilution of Primary Antibody)

Secondary Antibody, diluted 1:300 directly in 0.05% Saponin/PBS

Volume of antibody/cover slip = 30µl for 18 mm square
                             20µl for 11mm square
                             8µl for ovoid (round)

3% PFA and Mowiol Mounting Medium, see Immunofluorescence – Triton

Procedure

1. Place coverslips in individual wells of multi-well plate. Grow cells to desired confluence and perform experiment.

2. Wash 1x with PBS(-), 2ml/well.

3. 0.5% Saponin/Pipes for 5 minutes, 2ml/well for a 6-well plate. Less is needed for a 12 or 24-well plate.

4. Remove Saponin/Pipes, then fix with 3% PFA for 15 minutes, 1ml/well, can be left on overnight @ 4°C. After fixation, never let the coverslips become dry.

5. Wash 1x with 0.05% Saponin/PBS(-) for 5 minutes, 2ml/well.

6. Remove Saponin/PBS(-), then add 50mM NH₄Cl/PBS(-) for 5 minutes (blocking agent), 2ml/well.

7. Wash 1x with 0.05% Saponin/PBS(-) for 5 minutes, 2ml/well.

8. Incubate with Primary Antibody in 0.05% Saponin/PBS(-) for 20 minutes.
9. Wash 3x 5 minutes with 0.05% Saponin/PBS(-), 2ml/well.

10. Do a quick spin of diluted antibody to remove clumps, then incubate with secondary antibody in 0.05% Saponin/PBS(-) for 20 minutes. *

   * If secondary antibody is biotinylated and requires detection with Streptavidin-Texas Red, then:
     a) Wash 3x 5 minutes with 0.05% Saponin/PBS(-).
     b) Incubate with Streptavidin conjugate in 0.05% Saponin/PBS(-) for 20 minutes.
     c) Proceed with steps 10-12.

11. 0.05% Saponin/PBS(-) for 5 minutes, 2ml/dish

12. Wash 3x 5 minutes with PBS(-), 2ml/dish

13. Blot top and edges of coverslip with filter paper and mount coverslip on Mowiol, about 20µl for an 18 mm coverslip.