Immunofluorescent Staining Protocol

Immunocytochemistry

A. **Solutions and Reagents**
   1. 1X Phosphate Buffered Saline (PBS): Dissolve 8g NaCl, 0.2g KCl, 1.15g Na2HPO4 and 0.2g KH2PO4 in 800mL distilled water (dH2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature
   2. Poly-L-lysine solution: 0.1mg/ml in 1xPBS
   3. Glass coverslips No.1, 18mm dia.
   4. Fixation buffer: 4% paraformaldehyde in 1xPBS
   5. Permeabilization buffer: 0.1% Triton X-100 in 1xPBS
   6. Blocking buffer: 5% Fetal Bovine Serum (FBS) in 1xPBS
   7. Fluorescence-labeled secondary antibody

B. **Fixation permeabilization**
   1. Coat the coverslips with 0.1mg/ml poly-L-lysine solution at room temperature for 2hrs, dry, and then wash with 1xPBS buffer.
   2. Place the coated coverslip into each well of 12-well plate, and inoculate cells the day before immunocytochemistry experiment.
   3. Suck off the medium and rinse cells attached to cover slips twice with 1xPBS, removing liquid by gentle aspiration in this and subsequent steps.
   4. Fix cells with 4% paraformaldehyde in 1xPBS for 6 min at room temperature, and then rinse briefly twice with 1xPBS.
   5. The fixed cells can be permeabilize with 0.1% Triton X-100 in 1xPBS for 6 min.
   6. Wash cells briefly twice with 1xPBS, then block the coverslip with blocking buffer briefly at R/T.

C. **Staining**
   1. Dilute primary antibody with blocking buffer, and incubate the coverslip for 60 min at room temperature.
      Note: You may wish to leave one slip for a secondary antibody only control.
   2. Wash cells 3 times with 1xPBS, then 2 times with blocking buffer.
   3. Incubate cells with a dilution of the fluorescence-labeled secondary antibody in blocking buffer for 30–45 minutes at room temperature in the dark.
   4. Wash cells three times with 1xPBS.

Mount the coverslip on a glass slide. Store the slides in the dark.