Immunofluorescence Protocol

A. Soultions and Reagents

1. **10× PBS:** To prepare 1L add 80g NaCl, 2g KCl, 2g KH_2PO_4 and 28.5g $NaHPO_4$ to 1L water for injection. Adjust pH to 7.4.
2. **4% Polyoxymethylene:** To prepare 100mL add 4g polyoxymethylene to 100mL 1×PBS. Adjust pH to 7.4.
3. **1×PBS/0.2% Triton X-100(PBS/Triton):** To prepare 500mL add 1mL Triton X-100 to 500mL 1× PBS.
4. **1×PBS/3% BSA(PBS/BSA):** To prepare 100mL add 3g BSA to 100mL 1× PBS.

B. Preparation Fixation permeabilization

1. Rinse cells briefly in PBS.
2. Aspirate PBS, cover cells to a depth of 2-3mm about 200ul with 4% polyoxymethylene.
3. Allow cells to fix for 15 minutes at room temperature.
4. Aspirate fixative, rinse three times in PBS for 5 minutes each.
5. Aspirate PBS, cover cells to a depth of 2-3mm about 200ul with PBS/Triton for 5 minutes at room temperature.
6. Aspirate permeability agent rinse three times in PBS for 5 minutes each.

C. Immunostaining

1. Gently add 200ul of primary antibody diluted in PBS/BSA to the 24 well plates each well.
2. Incubate 60 minutes at 37℃ or overnight at 4℃.
3. Aspirate diluted primary antibody, then rinse three times in PBS for 5 minutes each.
4. Incubate in fluorochrome-conjugate secondary antibody diluted in PBS/BSA to the 24 well plates 100ul each well for 30 minutes at room temperature in dark.
5. Aspirate diluted fluorochrome-conjugate secondary antibody, then rinse three times in PBS for 5 minutes each.
6. Test under fluorescence microscope.