Immunofluorescence Protocol

All steps in this protocol are performed at room temperature unless otherwise indicated. For optimum staining, incubations should be carried out on a slow-moving rotary shaker unless the cell line being used is delicate.

1. Aspirate medium, wash cells seeded on clean glass cover slips briefly with 30%-40% coverage. When they reach 80-90% coverage, wash quickly with 500 ul of 1XPBS, for 3 times.
2. Fix the cells with 4% paraformaldehyde made fresh in 1X PBS for 10 minutes. Wash cover slips with 1X PBS 3 times for 5 minutes each.
3. Permeabilize the cells with 0.1% to 0.5% Triton X-100 made in 1X PBS for 10-30 minutes. Wash cover slips 3 times with 1X PBS for 5 minutes each.
4. Block the cells with the blocking buffer (10% FBS, 1% BSA in 1XPBS) for 30 minutes.
5. Apply primary antibody in blocking buffer at 1:50 to 1:1000 for 2 hours at room temperature. Or, alternatively, incubate overnight at 4°C.
6. Wash the cells with 0.1% Tween 20 in 1XPBS 3 times for 10 minutes each.
7. Apply an appropriate fluorophore-conjugated secondary antibody in blocking buffer at 1:50 to 1:1000 and DAPI at 1:2000.
8. Wash the cells with 0.1% Tween 20 in 1XPBS 3 times for 10 minutes each.
9. Examine cover slips under a fluorescence microscope. Take images.