

## Immunofluorescence Microscopy Protocol

### Sample preparation:

1. Grow cultured cells on chamber slides overnight, or add appropriate amount of cells to poly-L-lysine coated chamber slides and incubate at least 30 min at 37°C, at the time of fixation cells should be ~50% confluent.
2. Rinse cells briefly in PBS.
3. Fix cells by incubation with 4% Paraformaldehyde, in PBS for 15 min at room temperature.
4. Rinse three times in PBS, 3 min each.
5. Add ice-cold acetone and incubate at -20°C for 10 min.
6. Rinse three times in PBS, 3 min each.

### Sample Blocking:

Block samples in 5% normal serum from same species as secondary antibody in 1% BSA/0.2% Triton X-100/PBS for 1 h at room temperature, or overnight at 4°C.

### Sample staining:

7. Dilute the primary antibody to the recommended concentration/dilution in 1% BSA/0.05% Triton X-100/PBS.
8. Add 200 µl per well (8 wells) to the chamber slides and incubate 2 h at room temperature, or overnight at 4°C.
9. Rinse three times in PBS, 3 min each.

**NOTE: If using primary antibodies directly conjugated with FITC or Alexa Fluor® 488, then skip to step 13.**

10. Prepare fluorochrome-conjugated secondary antibody antibodies in 1% BSA/0.05% Triton X-100/PBS according to the recommended manufacturer specification data sheet and add 200 µl per well (8 wells) to the chamber slides.
11. Incubate the samples for 1 h at room temperature in dark.
12. Rinse three times in PBS, 3 min each.
13. Coverslip with anti-fade mounting medium.
14. Seal slides with nail polish.

### **Solutions and Buffers**

#### **Phosphate Buffered Saline (PBS):**

8.0 g NaCl

1.44 g Na<sub>2</sub>HPO<sub>4</sub>

0.24 g KH<sub>2</sub>PO<sub>4</sub>

0.2 g KCl

Add ddH<sub>2</sub>O up to 1 L, pH to 7.2 with HCl

#### **1%BSA/PBS:**

1 g of BSA in 100 ml PBS