



## **Immunofluorescence Protocol for Adherent Cells on Microscopy Slide -Methanol Fixation-**

1. Plate approximately 200  $\mu$ l of cell suspension into each well of a microscopy slide (Cell line).
2. Incubate for 24 hours in a 37 °C CO<sub>2</sub> incubator.
3. Carefully aspirate culture medium and rinse cells carefully with phosphate buffered saline (PBS).
4. Add ice-cold methanol for 5 minute at room temperature.
5. Immediately wash cells twice for 5 minutes with phosphate buffered saline (PBS).
6. Cover cells with 8 % BSA in PBS and incubate for 1 hour at room temperature. Perform the incubation in a sealed humidity chamber to prevent air-drying of the cells.
7. Wash cells twice for 5 minutes with PBS.
8. Gently remove excess PBS and cover cells with primary antibody of choice diluted in 1 % BSA in PBS. Replace the lid of the humidity chamber and incubate for 0.5 to 2 hours at room temperature.
9. Wash cells three times for 5 minutes with PBS.
10. Gently remove excess PBS and incubate cells with a fluorescein-conjugated secondary antibody of choice diluted in 1 % BSA in PBS for 0.5 to 2 hours at room temperature in the dark. Perform the incubation in a sealed, humidity chamber to prevent air-drying of the tissue sections.
11. Wash cells three times for 5 minutes with PBS in the dark.
12. Mount cover slip and examine specimen under fluorescent microscope.