**Immunofluorescence General Protocol**

**(Service provided by Shikhar Biotech)**

### Culture desired cell line in a 12 well culture plate each containing one cover slip each. The seed must contain 20 X 103 each and should be cultured according to its doubling time. Cells should be ready for processing 18-20 hours of culture.

## **Preparation of slides for microscopy**

### Wash cells with 1mM MgCl2 PBS for 3 x 5minutes.

### Fix cells with 4% PFA for 10 minutes at room temperature.

### Pipette out PFA and then permeabilize cells with 0.15% Triton X for 15 minutes at room temperature.

### Wash with 1mM MgCl2 PBS for 3 x 5minutes.

### Block with 10% serum (serum of secondary antibody host).

### Incubate cells with primary antibody (diluted e.g. 1:50 in 1% BSA in PBST -please refer to datasheet for recommended concentration) for 1 hour or overnight at 4oC.

### Decant solution and wash cells with PBST for 3 x 5minutes.

### Incubate cells with diluted secondary antibody in 1% BSA in PBS for 1 hour at room temperaturein dark.

### Wash cells with PBST for 3 x 5minutes in dark.

1. Incubate cells with 1:1000 DAPI for 5 minutes in dark.

### Wash cells with PBST for 3 x 5minutes in dark.

### Take a drop of DPX in a slide and then mount the cover slip\* on an upside down on top of DPX. \*Recommended to use Shi-FixTM coverslips: http://everestbiotech.com/shi-fix-coverslips/

### When the cover slip is dry seal it with transparent nail polish.

1. Observe under Fluorescence Microscope.