Immunofluorescence General Protocol

(Service provided by Shikhar Biotech)

1. Culture desired cell line in a 12 well culture plate each containing one cover slip each. The seed must contain 20 X 10^3 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

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

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

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

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

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

7. 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 4^{o}C.

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

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

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

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

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

13. 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-Fix™ coverslips: http://everestbiotech.com/shi-fix-coverslips/

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

15. Observe under Fluorescence Microscope.