# Immunocytochemistry Protocol

## MATERIALS

4% PFA fixed cell arrays
Coverslips
Slide racks
Staining dishes with lids
Plastic slide tray (Baxter Scientific Cat. No. M6304)
Orbital shaker
Transfer pipettes

Deionized water (DI $H_2O$)
PBS (Phosphate Buffered Saline)
Triton X-100
Hydrogen peroxide ($H_2O_2$)
Primary antibody
Biotinylated secondary antibody, HRP conjugated
Bovine Serum Albumin (BSA – for blocking)
Streptavidin-HRP
DAB
Hematoxylin (optional)
Acetic Acid (optional)
Glycerol

## **Permeabilize Membrane** (Optional if detecting a membrane protein.)

1. Add one drop of PBS/0.1% Triton X-100 to each well to permeabilize the cells. Incubate slides for one (1) minute at room temperature.
2. Remove the liquid and wash the slides twice (2x) in PBS, 5 minutes each on the shaker.
3. Remove the liquid and place the slides onto a tray.

## Blocking

4. Soak slides in 1.5% $H_2O_2$ /PBS solution for 15 minutes.
5. Wash twice (2x) in PBS for 5 minutes each on the shaker.
6. Incubate with 5% BSA into each well to block for overnight at 4°C in a humid chamber.

## Primary Antibody

7. Dilute the primary antibody to the recommended concentration in 1% BSA diluent.
8. Remove BSA from the slides.
9. Add 35µL of primary antibody to each well. Incubate for one (1) hour at room temperature.
10. Remove the primary antibody solution and wash slides three (3) times in PBS, 5 minutes each on the shaker.

## Secondary Antibody and Detection

11. Dilute the biotinylated secondary antibody to 1:200 in a solution of 1% BSA diluent.
12. Remove the excess fluid and add one drop secondary antibody solution into each well. Incubate for one (1) hour at room temperature.
13. Wash in PBS three (3) times 5 minutes each on an orbital shaker. Remove excess fluid.
14. Add one drop streptavidin-HRP to each well. Incubate for 30 minutes at room temperature.
15. Wash three (3) times 5 minutes in PBS on an orbital shaker. Remove excess fluid.
16. Add DAB solution to each cell well. Once the cells start turning brown (inexperienced technicians may wish to observe this under a microscope) wash twice (2x) in PBS for 5 minutes each time on the shaker.

## Optional Counterstain

17. Dip the slide rack with the slides into a staining dish of hematoxylin for 30 seconds.
18. Remove and place into an acid bath (200mL DI $H_2O$ and one to three drops of acetic acid). Rinse with DI $H_2O$.

## Cover Slips

19. Add several drops of coverslip solution (50% glycerol / DI $H_2O$) to the slide.
20. Place the coverslip on top of the slide.
21. Store slides at room temperature.