ROCKLAND

Immunocytochemistry
Protocol

I. Materials & Reagents

Product	Preparation
Phosphate Buffered Saline (PBS)(MB-008)	
Triton X-100	
Hydrogen Peroxide (H_2O_2)	
Bovine Serum Albumin (BSA)	
Biotinylated Secondary Antibody, HRP-Conjugated	Dilute 1:500
Streptavidin Peroxidase Conjugated (S000-03)	Dilute 1:500
3,3'-Diaminobenzidine (DAB) Substrate (DAB-10)	
Hematoxylin (Optional) and Acetic Acid (Optional) for counterstain	
UltraPure Sterile Water (MB-009-1000)	
Coverslip Solution	50% glycerol/Ultrapure water

II. Procedure

Note: This procedure is optional if detecting a membrane protein.

1. Add one drop of PBS/0.1% Triton X-100 to each well to permeabilize the cells and incubate slides for one 1 minute at room temperature.
2. Remove the liquid and wash the slides twice in PBS, 5 minutes each on the shaker.
3. Remove the liquid and place the slides onto a tray.
4. Soak slides in 1.5% H_2O_2/PBS solution for 15 minutes.
5. Wash twice 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.
7. Dilute the primary antibody to the recommended concentration in 1% BSA diluent.
8. Remove BSA from the slides.
9. Add 35 µL of primary antibody to each well. Incubate for one 1 hour at room temperature.
10. Remove the primary antibody solution and wash slides 3 times in PBS, 5 minutes each on the shaker.
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 3 times, 5 minutes each on an orbital shaker. Remove excess fluid.
14. Add one drop streptavidin peroxidase to each well. Incubate for 30 minutes at room temperature.
15. Wash 3 times, 5 minutes in PBS on an orbital shaker. Remove excess fluid.
16. Add DAB substrate to each cell well. Once the cells start turning brown wash 2 times in PBS for 5 minutes each on the shaker.
    Note: Inexperienced technicians may wish to observe cells turning brown under a microscope.
17. (Optional step for counterstain) Dip the slide rack with the slides into a staining dish of hematoxylin for 30 seconds. Remove and place into an acid bath (200 mL UltraPure water and 1–3 drops of acetic acid). Rinse with UltraPure water.
18. Add several drops of coverslip solution.
19. Place the coverslip on top of the slide.
20. Store slides at room temperature.