ROCKLAND

Immunohistochemistry (IHC) Protocol

I. Reagents and Equipment Required

Reagent/Equipment
Phosphate Buffered Saline (PBS)(MB-008)
Xylene
95% and 100% Ethanol
UltraPure Sterile Water (MB-009-1000)
Antibody Dilution Buffer: Prepare 100 mL of PBS, supplemented with 1 mL of normal serum of same species as host used for the secondary antibody.
30% Hydrogen Peroxide Solution (KHJ001)
Biotinylated Secondary Antibody, 1:500 (e.g. 611-106-122)
Streptavidin Peroxidase Conjugated, 1:500 (S000-03)
DAB Substrate (DAB-10) or TMB Membrane Peroxidase Substrate (TMBM-100) for stable brown or blue staining, respectively.
Polymount Mounting Media (KHH001)

II. Procedure for Frozen Sections

1. Snap-freeze fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store frozen blocks at -80°C.
2. Cut cryostat sections 4–8 mm thick and mount onto Superfrost™ Plus slides or gelatin-coated slides. Store slides at -80°C until needed.
3. Before staining, warm slides at room temperature for 30 minutes and fix in ice-cold acetone for 10 minutes. Air-dry for 30 minutes.
4. Wash in PBS.

III. Procedure for Paraffin Sections

1. Deparaffinize sections in xylene 2 times for 5 minutes.
2. Hydrate with 100% ethanol 2 times for 3 minutes.
3. Hydrate with 95% ethanol for 1 minute.
4. Rinse in UltraPure sterile water.

IV. Procedure for Immunoenzyme Staining

1. Follow procedure for pretreatment as required.
2. Rinse sections in PBS 2 times for 2 minutes.
3. Incubate sections in normal serum block with the same species as the secondary antibody (e.g., Normal Goat Serum (NGS) (B-304) if secondary antibody is goat host).
   Note: Since this protocol uses avidin-biotin detection system, avidin/biotin block may be needed based on tissue type. If you do, the avidin/biotin blocking should be done after normal serum block and before primary antibody incubation.
4. Incubate sections in primary antibody at appropriate dilution in dilution buffer for 1 hour at room temperature or overnight.
   Note: Do not rinse sections between serum block and primary antibody incubation.
5. Rinse in PBS buffer for 3 times for 2 minutes.
6. Incubate sections in 1% hydrogen peroxidase in PBS for 10 minutes at room temperature.
7. Rinse in PBS buffer 3 times for 2 minutes.
8. Incubate sections in biotinylated secondary antibody in PBS buffer for 30 minutes at room temperature.
9. Rinse in PBS buffer 3 times for 2 minutes.
10. Incubate sections in streptavidin peroxidase in PBS buffer for 30 minutes at room temperature.
11. Rinse in PBS buffer 3 times for 2 minutes.
12. Incubate sections in peroxidase substrate solution.

13. Rinse in PBS buffer 3 times for 2 minutes.
14. Rinse in UltraPure sterile water 3 times for 5 minutes.
15. Dehydrate through 95% ethanol for 1 minute, 100% ethanol 2 times for 3 minutes.
16. Clear in xylene 2 times for 5 minutes.
17. Coverslip with mounting medium (KHH001).