ROCKLAND™
antibodies & assays

Staining Paraffin Sections by PAP Procedure

(Mouse Monoclonal Antibodies)

1. Deparaffinize sections by sequential immersion in the following for 2' each: xylene (twice); 50% xylene/50% ethanol; absolute ethanol (twice), 95% ethanol (twice), 70% ethanol. Agitate gently in each solution. If section is from non-perfused tissue, treat for 30' with 3% hydrogen peroxide in methanol.

2. Rinse three times in "1.5T buffer" which consists of 0.05 M Tris, pH 7.6 supplemented with 1.5% (w/ v) Sodium Chloride.

3. Put a small quantity of distilled water in the 4 quarters of a divided Petri dish and place a slide on top of the dividing ridges. Petri dishes should be covered throughout the staining procedure to provide a humidified atmosphere.

4. Prevent the spreading of reagents on the slide off the confines of the sections by placing a ring around the section using a Pap Pen (Code # KHP001) or equivalent method. Never let sections themselves dry.

5. Quickly thaw normal goat serum (NGS) (Code #B304) in a 37° water bath. Mix, but do not shake or vortex.

6. Cover Sections with 3% NGS in 1.5T buffer (such as 0.1 ml of NGS + 2.9 ml of 1.5T buffer). Always make up only the amount needed, assuming 25 to 50 ml per section. Incubate at room temp for 30'.

7. Obtain monoclonal antibodies from freezer. Thaw quickly, mix gently but do not shake or vortex. Place in ice bucket. Make antibody dilutions in 1.5T buffer containing 1% NGS (for instance, a 1:1000 dilution by adding to 2 ml of antibody, 1,998 ml of 1.5T buffer with1% NGS).

8. Shake off NGS and rim sections. Apply antibodies. Incubate at room temp or in refrigerator overnight (incubation times may vary from 30' to over a weekend.)

9. Dilute goat anti-mouse IgG 1:100 in 1.5T buffer.

10. Squirt sections with 1.5T buffer and rinse in three changes of 1.5T buffer. Apply diluted goat antimouse IgG (H&L) [Code # 610-4102] for 30' at room temperature.

11. Dilute PAP 1:100 in 1.5T buffer with 1% NGS (for instance, 10 ml of PAP + 1.99 ml of 1.5T buffer).

12. Squirt sections and rinse in three changes of 1.5T buffer. Rim. Apply PAP. Leave at room temp for 30'. Keep remaining diluted PAP.

13. Prepare diluted diaminobenzidine tetrahydrochloride (DAB) with hydrogen peroxide added. It is convenient to store frozen 0.5 ml volumes of 10X DAB (Code # DAB-10). This stock solution is made by dissolving 100 mg DAB in 20 ml of 1.5 T. Immediately before use, add to0.5 ml of stock DAB, 4.5 ml of 1.5 T followed by 2.7 ml of 30% hydrogen peroxide (use gloves). Test DAB solution by adding a few drops to the diluted PAP tube kept in step 12.

14. Squirt sections and rinse three times in 1.5T buffer. Add DAB/H2O2 solution. Incubate for approximately 8'.

15. Rinse in 1.5T buffer three times.

16. Dehydrate (reverse of step 1 and cover with Polymount Mounting Media (Code # KHH001) in fume hood.