ROCKLAND

Staining Paraffin Sections by PAP Protocol

(Mouse Monoclonal Antibodies)

Procedure

- Deparaffinize sections by sequential immersion in the following for 2 minutes 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 minutes with 3% hydrogen peroxide in methanol.
- 2. Rinse three times in 0.05 M Tris, pH 7.6 supplemented with 1.5% (w/v) Sodium Chloride (1.5T buffer).
- 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. Place a ring around the section using a PAP PEN (KHPOOI) (or equivalent method) to prevent the spreading of reagents on the slide. Do not allow the section to dry completely.
- 5. Quickly thaw Normal Goat Serum (NGS) (B304) in a 37°C 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 25–50 mL per section. Incubate at room temp for 30 minutes.
- 7. Thaw monoclonal antibodies quickly on ice and mix gently but do not shake or vortex. 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 with 1% NGS).
- 8. Shake off and apply antibodies. Incubate at room temp or in refrigerator overnight (incubation times may vary from 30 minutes to over a weekend.)
- 9. Dilute goat anti-mouse IgG 1:100 in 1.5T buffer.
- **10.** Gently rinse sections with 1.5T buffer three times. Apply diluted goat anti mouse IgG (H&L)(610-4102) for 30 minutes 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. Gently rinse sections with 1.5T buffer three times. Apply PAP. Leave at room temperature for 30 minutes. Keep remaining diluted PAP for following step.
- 13. Prepare diluted diaminobenzidine tetrahydrochloride (DAB) with hydrogen peroxide added. It is convenient to store frozen 0.5mL volumes of 10X DAB (DAB-10). This stock solution is made by dissolving 100 mg DAB in 20 mL of 1.5 T buffer. Immediately before use, add to 0.5 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. Gently rinse sections with 1.5T buffer three times. Add DAB/H_2O_2 solution. Incubate for approximately 8 minutes.
- 15. Rinse in 1.5T buffer three times.
- 16. Dehydrate (reverse of step 1) and cover with Polymount Mounting Media (KHHOO1) in fume hood.

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