Staining Paraffin Sections by PAP Protocol

(Mouse Monoclonal Antibodies)

Procedure

1. 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 (KHP001) (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 (KHH001) in fume hood.