Immunohistochemistry

I. Preparation

A. Cryostat sections
1. Cut 4µM sections and pick up on coverslips.
2. Fix in ice-cold acetone for 2 minutes.
3. Air dry.
4. Using a hydrophobic pen (PAP-PEN©) draw a ring around the section
5. Rehydrate sections in PBS for 5 minutes. From this point on do not allow sections to dry out.

B. Cultured cells
1. Plate cells on sterile coverslips or in cell chambers (Note: If detecting with a fluorescent label always use glass) at desired confluence 12 - 24 hours before staining.
2. Wash cells 3 changes of PBS.
3. Fix cells in ice cold acetone for 2 minutes.
4. Air dry.
5. If possible use a hydrophobic pen (PAP-PEN©) to outline the coverslip.
6. Rehydrate cells in PBS for 5 minutes. From this point on do not allow cells to dry out.

II. Immunostaining

1. Dilute primary antibody to appropriate concentration in PBS- 1%BSA. Use approximately 50- 150µL diluted antibody per coverslip
2. Prepare a humidified chamber by lining a 150mm culture dish with a layer of filter paper and soak it with PBS.
3. Place coverslips tissue side up (taking notice of the order) in the humidified chamber.
4. Incubate with primary antibody at room temperature for 1 hour.
5. Rinse in three changes of PBS, 5 minutes each.
6. Dilute secondary antibody to appropriate concentration in PBS.
7. Incubate for 45 minutes at room temperature.
8. Rinse in three changes of PBS, 5 minutes each.
9. If secondary antibody was fluorescence-conjugated proceed to mounting.
10. If secondary antibody was biotin conjugated prepare streptavidin conjugate in the appropriate concentration in PBS.
11. Incubate for 30 minutes at room temperature.
12. Rinse in three changes of PBS, 5 minutes each.
13. If streptavidin was conjugated with a fluorescent label proceed to mounting.
14. If streptavidin was conjugated with horseradish peroxidase prepare DAB solution.
15. Incubate in DAB solution for 5 minutes. Monitor under microscope and return to DAB solution until desired stain is reached.

DAB solution: 5mg DAB
100 mL MilliQ water
0.1mL 0.3% H_2O_2

III. Mounting

A. Fluorescence
1. Rinse in three changes of distilled water and mount on slides with Farrant's medium.

B. DAB
1. Rinse in three changes of distilled water, dehydrate through three changes of 100% alcohol, and clear through three changes of xylene (use fume hood).
2. Mount on slides using Permount.