Immunohistochemistry Protocol Alpers lab 2/26/01

Materials:

Phosphate buffered saline (PBS), pH 7.4
PBS with BSA (use globulin free BSA, Sigma # A7638)
Tris buffered saline (TBS)
3% Hydrogen peroxide
Xylene
Ethyl alcohol – 100%, 95%, 75%
ABC Standard Kit (Vector #PK4000)
Diaminobenzidine (DAB) (Sigma #D5637)
Nickel Chloride, 8% solution
Methyl green
Histosolve (Shandon #9990505)
Xylene substitute mountant (Shandon #1900233)

Possibly needed for antigen retrieval:
Trypsin IX (Sigma #T1034)
Pronase (Cal – Biochem #53702)
Antigen Unmasking Solution (Vector #H-3300)

Method:

The following method is meant as a guideline for using the ABC technique. Each laboratory will need to alter specific times and dilutions to meet the specific needs of their antibodies.

1. Label all slides with pertinent information such as method of fixation, antibody dilution, enzymatic treatment, etc.

2. Place slides in racks. If doing a large number of slides, group slides which will be receiving the treatment (eg. Same dilution of primary antibody)

3. Deparaffinize in three changes of xylene – 5 minutes each

4. Rehydrate – 100% EtOH- 3 changes, 5 minutes total
   95% EtOH- 2 changes, 4 minutes total
   75% EtOH- 1 changes, 1 minutes total

5. Block endogenous peroxidase by placing slides in 3 % hydrogen peroxide for 5 minutes (you can also use 10 minutes in 0.1% sodium azide and 0.3% hydrogen peroxide). You may skip this step if the tissue you are working with does not have endogenous peroxidase activity.

6. Wash in one change of PBS for 5 minutes.

7. Incubate slides requiring antigen retrieval (enzyme or heat) in appropriate solution required. The specific times/enzymes will need to be worked out for each primary antibody. Excessive or inadequately unmasked antigen, respectively.

8. If you experience a great deal of background, particularly if your primary antibody is made in rabbit, then the tissue section must be incubated in normal goat serum to prevent non-specific binding of the secondary goat-anti-rabbit antibody. Incubate for 10 minutes. Do not wash slides. Suction or wipe away as much of the NGS as possible without letting the tissue section dry out.

9. Apply enough of the appropriately diluted primary antibody (diluted in PBS/BSA) to cover the tissue and incubate 60 minutes in a moist chamber at room temperature (RT). You may also incubate the slides overnight in a moist chamber at 4 °C (in the refrigerator).

10. Wash in two changes of PBS – 10 minutes each

11. Apply biotinylated secondary antibody (diluted as per kit instructions – 1:300 in PBS/BSA). Incubate 30 minutes at RT in moist chamber. Dilution may be changed if staining is inappropriate.

12. Make up ABC complex (in PBS/BSA), as it needs to be made at least 30 minutes in advance (add 15 ul A and 15 ul B to each ml. of PBS/BSA)

13. Wash in two changes of PBS – 10 minutes each

14. Apply ABC and incubate 30 minutes at RT in moist chamber.

15. Wash in two changes of PBS – 10 minutes each

16. To 175 ml buffer, add 4 ml thawed DAB, 1 ml 8%NiC12 – mix prior to use and keep at 37°C. Just before use, add12 drops 3% hydrogen peroxide and mix.

17. Incubate slides in DAB solution for 10 minutes at 37 °C.

18. Wash in dH_2O – one change, 2 minutes.

19. Counterstain in methyl green for 1 minute.

20. Rinse in 95% EtOH (a few quick dips).

21. Rinse in 2 changes of 100% EtOH (a few quick dips in each)

22. Clear in Histosolve – 3 changes for 5 minutes total and coverslip with Xylene substitute mountant.