Immunohistochemistry for paraffin sections

(prepared by P. Sun)

Materials:

- Permount (Fisher, Cat#SP15-100)
- Sodium citrate (Fisher, Cat#S279-3)
- 30% H_2O_2 (Fisher, Cat#H325-500, store at -20C)
- Biotinylated anti-mouse IgG (Vector Lab. Cat#BA-2001)
- Biotinylated anti-rabbit IgG (Vector Lab. Cat#BA-1000)
- ABC kit (Vector Lab. Cat#PK-6100)
- DAB (Dako, Cat#2012-08)
- BSA (Fisher, Cat#BP1605-100)

Solutions

- Antigen retrieval
  sodium citrate ---------- 2.94 g
  Distilled water ---------- 1000 ml
  Mix to dissolve. Adjust pH to 6.0 with 1N HCl and mix well. Store this solution at room temperature for 3 months or at 4 C for longer storage.
- ABC solution
  Prepare 30 min before by mixing: 20µL of solution A, 20µL of solution B, 960 µL of 1X PBS.
- DAB solution
  Mix 25 ul of CHROMOGEN and 1 ml of SUBSTRATE BUFFER

For additional details – consult manufacturer booklets for ABC and DAB.

1. Deparaffin: xylene 2 x 5 min at RT
2. Hydrate at RT
   (1) 100% EtOH 2 x 3min
   (2) 95% EtOH 1 x 2min
   (3) 70% EtOH 1 x 2min
   (4) 50% EtOH 1 x 2min
   (5) 30% EtOH 1 x 2min
   (6) 1 x PBS 2 min
3. Antigen retrieval
   (1) Heat section in 10 mM sodium citrate buffer (pH6.0) for 10 min in microwave placed in the lab.
   (2) Cool to RT, wash once with 1 x PBS for 2 min
4. Quench endogenous peroxidase activity
   (1) Incubate section in 3% H_2O_2 (30% H_2O_2 is diluted in water at 1:10) for 5 min at RT
   (2) Wash 3 x 5 min in 1 x PBS at RT.
5. Circle sections (6-8 um) with PAP PEN.

6. Block with blocking buffer (5-10% BSA in 1 x PBS) for 30 min at RT
7. Incubate with primary antibody at 4°C for ON: primary antibody in dilution buffer (1-2% normal serum in 1 x PBS)
8. Wash 3 x 5 min in 1 x PBS at RT.
9. Incubate with secondary antibody for 1 h at RT: Biotinylated anti-mouse IgG or Biotinylated anti-rabbit IgG diluted 1:200 in in dilution buffer (1-2% BSA in 1 x PBS).
10. Wash 3 x 5 min in 1 x PBS at RT.
11. Incubate with ABC solution for 45-60 min at RT
12. Wash 3 x 5 min in 1 x PBS at RT.
13. Wash 2 x 5 min in 1 x TBS at RT.
14. Incubate with DAB solution (color development should be monitored under microscope; make sure not over-developed to minimize background signals)
15. Rinse with H_2O for 5 min at RT
16. Rinse with 1X PBS for 5 min at RT
17. Mount slides with Permount