

Imuno-Staining For CD-31 (Pharmingen #01951D rat anti-mouse CD-31)**PROCEDURE:****A) Unfixed tissue, embedded in OCT and frozen at -80C. Cut Frozen sections @ 50u thick**

Remove slides from -80 °C and bring to RT; allow to dry completely

Peel off OCT from around sections carefully

Acetone Fix in 100% acetone @4°C 10 minutes

Dry slides completely

B) Staining:

1. Using PAP pen, "draw" around sections carefully
2. Rehydrate slides in PBS 5-10 minutes RT
3. Apply Avidin block 15 minutes @ RT (Vector SP-2001)
4. PBS wash 2x 3 minutes @ RT
5. Apply Biotin block 15 minutes @ RT (Vector SP-2001)
6. PBS wash 2x 3 minutes @ RT
7. Block sections in Blocking buffer (5% normal goat serum, 2.5% BSA, 1X PBS) for 30-45 min in humidified chamber @ RT (use about 150-200ul/section)
8. Aspirate block
9. Apply Primary antibody 1:75 (diluted in 0.5X blocking buffer) to section. Incubate in humidified chamber @ RT 2 hours. (Lot # used _____)
10. Aspirate antibody
11. PBS wash 3x 3min.
12. Block slide in Blocking buffer (as in step 7) for 5-10 min. @ RT
13. Apply biotinylated secondary goat anti-rat (Pierce #31831) at ~1:200 (dilute in 0.5X block buffer) to sections for 30-45 min. @ RT in humidified chamber. (Lot # used _____)

***Make up ABC-Elite** according to manufacturer's specifications

(2 drops ABC-A in 5 ml 0.5M NaCl, vortex, 2 drops ABC-b, vortex, let sit 30 minutes).

14. PBS 2 x 3 min.
15. Block endogenous peroxidase activity in 0.3 % H₂O₂ at RT for 10 minutes (2.5 ml 30 % H₂O₂ 247.5 ml PBS)
16. PBS 3x3 min
17. Apply ABC to sections and cover in humidified chamber at RT for 30 min.

*Make up DAB according to manufacturers instructions (Sigma #D-4418)

***EXTREME CARCINOGEN-WEAR GLOVES**

-Filter through 0.2µ filter/syringe

18. Wash slides in 0.1 M Tris pH 7.4 3 x 3 min.
 19. Apply DAB to sections and monitor time of development and record
 20. Wash in H₂O briefly x2
 21. Counterstain in 1% Methyl green 1min15sec @RT
 22. Water rinse Well
 23. **Dehydrate**
 - 100% Isobutanol 20 dips
 - 100% Isobutanol 2x 2 min
 - Xylene 2x 2 min _____
- Cover section while moist with Cytoseal #60