

Immunohistochemistry Protocol for Paraffin-Embedded Sections
PECAM Antibody Staining

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1. Xylene 5 min
2. Xylene 5 min
3. 100% Ethanol 5 min
4. 95% Ethanol 5 min
5. 70% Ethanol 5 min
6. 50% Ethanol 5 min
7. ddH₂O 5 min
8. 1X PBS (pH 7.8) 5 min
9. 3% H₂O₂ (30% grade) in Methanol 20 min (room temp)
 - ✓ Prepared from 30% H₂O₂ (Fisher, Catalog #H325-500)
10. 1X PBS 5 min
11. Proteinase K (0.1 mg/ml) 10-15 min (room temp)
 - ✓ Time dependent on the type and thickness of tissue
 - ✓ For most tissue 5 µm or less, 10 min incubation is sufficient
 - ✓ For more impenetrable tissue, incubate up to 15 min and at 37^o C as needed
12. 1X PBS 5 min
13. 1X PBS 5 min
14. Blocking Buffer 1 hour (1X PBS + 0.05% Tween + 1% serum)
 - ✓ Customize type of serum with specific secondary antibody of your choice
15. 1X PBS 5 min
16. Primary antibody overnight (4^o C, prepared in blocking buffer)
 - ✓ Purified rat anti-mouse CD31 1:100 (BD Pharmingen, Catalog #553370)
17. 1X PBS 3 min
18. 1X PBS 3 min
19. Blocking Buffer 10 min
20. Secondary antibody 1.5 hours, room temp
 - ✓ Biotinylated anti-rat IgG 1:150 (Vector Laboratories, Catalog #BA-4001 for antibody prepared in rabbit)
21. ABC Reagent Preparation Prepare & let stand for 30 min, room temp, prior to use
 - ✓ Vectastain ABC Kit (Vector Laboratories, Catalog #PK-6100)
 - ✓ 2 drops ABC-A in 5 ml 1X PBS, vortex;
 - ✓ 2 drops ABC-B, vortex.
21. 1X PBS 5 min
22. 1X PBS 5 min
23. ABC Reagent Usage 30 min, room temp
 - ✓ Depending on total area of tissue, add 300-400 µl ABC reagent
24. 1X PBS 5 min

25. DAB Solution Preparation
 - ✓ Peroxidase Substrate kit (Vector Laboratories, Catalog #SK-4100)
 - ✓ 2 drops buffer stock solution in 5 ml ddH₂O, vortex;
 - ✓ 4 drops DAB stock solution, vortex;
 - ✓ 2 drops hydrogen peroxide solution, vortex;
 - ✓ 2 drops nickel solution, vortex.
26. DAB Solution Usage 5-10 min, room temp
 - ✓ Depending on total area of tissue, add 300-400 µl DAB solution
 - ✓ When tissue section turns slightly brown, immerse slides in ddH₂O, wash thoroughly
 - ✓ May need to adjust DAB exposure time, depending on how intense staining appears
27. ddH₂O 5 min
28. Counterstaining 5-10 min
 - ✓ Nuclear Fast Red (Vector Laboratories, Catalog #H-3403)
 - ✓ Methyl Green (Vector Laboratories, Catalog #H-3402)
 - ✓ Or, counterstain color of your choice
29. ddH₂O 10 min, several changes
30. 50% Ethanol 1 min
31. 70% Ethanol 1 min
32. 95% Ethanol 1 min
33. 100% Ethanol 1 min
34. Xylene 1-10 min (depending on time needed to mount tissue)
35. Mount tissue
 - ✓ Permount (Fisher Laboratories, Catalog #SP15-500)
 - ✓ Add small drop sufficient to cover tissue, place coverslip gently on tissue