

Immunoperoxidase of Paraffin Embedded Sections

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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. dd H₂O 5 min
8. 1X PBS (ph7.8) 5 min
9. 3% H₂O₂ in Methanol 20 min – (made fresh - blocks endogenous peroxidases)
10. 1X PBS 5 min
11. Proteinase K (0.1 mg/ml) 5 min (stock 10mg/ml, 1:100 dilution)
12. 1X PBS 5 min
13. 1X PBS 5 min
14. Blocking Solution 1hr or o/n (1X PBS + 0.05% Tween + 1% serum)
15. 1X PBS 5 min
16. 1° Antibody (1:200) 1hr or o/n (make in 1X PBS + 1% serum)
17. 1X PBS 3 min
18. 1X PBS 3 min
19. Blocking Solution 7 min
20. 2° Antibody (1:200) 45 min

Prepare ABC reagents (1:250 dilution each in 1X PBS) and let it stand for 30 min at room temperature prior to use.

21. 1X PBS 3 min
22. 1X PBS 3 min
23. ABC Reagent 30 min
24. 1X PBS 5 min
25. DAB 5-10 min
26. dd H₂O 5 min
27. dd H₂O 5 min
28. Nuclear Fast Red Dye ~ 10 min (need to check slides)
29. dd H₂O ~ 10 min with running H₂O
30. 50% Ethanol 5 min
31. 70% Ethanol 5 min
32. 95% Ethanol 5 min
33. 100% Ethanol 5 min
34. Xylene 5 min
35. Xylene 5 min
36. Permount