



PRODUCT DESCRIPTION

FOR LAB RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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Protocol for Immunohistochemistry on Paraffin-Embedded Tissue Sections

1. Deparaffinize slides in 2 changes of xylene, one for 40 minutes, the other for 20 minutes.
2. Transfer slides to 100% alcohol, 95% alcohol, 80% alcohol, 60% alcohol and ddH₂O for 5 minutes each.
3. Place slides in vessel filled with antigen retrieval buffer and microwave on middle for 2x2 minutes (700 watt oven), allow retrieval solution to cool at room temperature.
4. Rinse 2 x 5 minutes with TBS.
5. Block endogenous peroxidase activity by incubating sections in 3% H₂O₂ solution in methanol for 30 minutes.
6. Rinse 2 x 5 minutes with TBS.
7. Block in 5-10% goat serum in TBS for 30 minutes at room temperature.
8. Drain slides for a few seconds (do NOT rinse) and wipe round sections.
9. Apply primary antibody made up in TBS. Incubate 1 hour at room temperature or 4°C overnight,
10. Rinse 2 x 5 minutes with TBST.
11. Apply secondary biotinylated antibody made up in TBS for 1 hour at room temperature.
12. Rinse 2 x 5 minutes with TBS.
13. Apply streptavidin peroxidase for 15 minutes or 30 minutes at room temperature.
14. Rinse 2 x 5 minutes with TBS.
15. Develop with chromogen (DAB) at room temperature, watching under microscope.
16. Rinse in running tap water for 5 minutes.
17. Counterstain in mayor's hematoxylin bath for 30-60 seconds.
18. Wash in water bath 7-8 times, then tap water for 3 minutes.
19. Dehydrate through 60%, 80%, 95% and 100% alcohol for 5 minutes each.
20. Transfer to xylene for 5 minutes. Air for 30 minutes.
21. Mounting.