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NBP1-79054 Protocol

IHC-P Protocol (NBP1-79054)

1. Deparaffinize the section in 3 changes of xylene, 5 minutes each.
2. Wash the section in 96%, 80% and 70% benzyl alcohol for 5 minutes each.
3. Rinse in distilled water.
4. Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H2O2) for 10 minutes.
5. Wash in distilled water.
6. For antigen retrieval: immerse the slide in Tris-EDTA buffer, pH 9.0, 0.05% Tween- 20*, and incubate in microwave (850W) for 20 minutes. (Alternatively adjust to your own protocol, keeping the required pH)
7. Remove the staining to room temperature and let the slide to cool for 15 minutes.
8. Rinse in distilled water.
9. Wash in 0.05 M Tris-HCl , pH 7.6 buffer supplemented with 0.2% of Tween-20 (buffer A) for 5 minutes.
10. Incubate the section with primary antibody diluted in buffer A at the dilution 1:100 - 200 for 1 hour in the closed wet chamber.
11. Wash twice 5 minutes with buffer A.
12. Apply the secondary antibody (the protocol depends on the supplier), and proceed to standard immunohistochemistry protocol (HRP - Peroxide - DAB).
13. Wash twice 5 minutes with buffer A.
14. Apply the chromogen (DAB), 10 minutes.
15. Wash in water - 10 minutes.
16. Stain in hematoxylin for 5 minutes.
17. Wash in water - 10 minutes.
18. Dehydrate the section in 2 changes of 96% benzyl alcohol for 5 minutes each.
19. Wash the section in 2 changes of xylene for 2 minutes each.
20. Mount the slide for observation.