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NBP1-33780 Protocol

Immunohistochemistry-Paraffin protocol for iNOS Antibody (NBP1-33780)

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https://www.novusbio.com/products/inos-antibody-k13-a_nbp1-33780
Immunohistochemistry-Paraffin

1. Deparaffinize the section in 3 changes of xylene, 5 minutes each.
2. Wash the section in 96%, 80% and 70% ethyl alcohol for 10 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 and incubate at 95-97C in water bath for 25 minutes. (Alternatively adjust to your own protocol, keeping the required pH)
7. Remove the staining to room temperature and let the slide to cool (in Tris-EDTA buffer, pH 9.0) for 15 minutes.
8. Rinse in distilled water, 2 x 5 minutes.
9. Wash in PBS (phosphate buffer saline, pH 7.0-7.5) supplemented with 0.05% of Tween-20 (buffer A), 2 x 5 minutes.
10. Incubate the section with primary antibody at the dilution 1:100 - 1:200 for 1 hour in the closed wet chamber.
11. Wash 3 x 5 minutes with buffer A.
12. Apply the secondary antibody (the protocol depends on the supplier), and proceed with immunohistochemistry protocol (HRP - Peroxide - DAB).
13. Wash 3 x 5 minutes with buffer A.
14. Apply the chromogen (DAB), 1-3 minutes.
15. Wash in water, 2 x 5 minutes.
16. Stain in hematoxylin for 5 minutes.
17. Wash in water, 2 x 5 minutes.
18. Stain in hematoxylin for 5 minutes.
19. Wash in distilled water, 3 x 2 minutes.
20. Mount the slide for observation.

* Tris-EDTA Buffer (10mM Tris Base, 1mM EDTA solution, pH 9.0)
Tris -- 1.21 g; EDTA -- 0.37 g; Distilled water -- 1000 ml
Mix to dissolve in 700 ml of distilled water. Adjust pH to 9.0 with 1M HCl and mix well.
Adjust the final volume to 1 liter with distilled water.
Store this solution at room temperature for 3 months or at 4C for longer storage.