Orders: orders@novusbio.com
Support: technical@novusbio.com
Web: www.novusbio.com
Protocols, Publications, Related Products, Reviews and more:
www.novusbio.com/NBP1-79069

NBP1-79069 Protocol

Immunohistochemistry-Paraffin protocol for Cytokeratin 14 Antibody (NBP1-79069)

IHC-P protocol (NBP1-79069): https://www.novusbio.com/products/cytokeratin-14-antibody-d19-n_nbp1-79069

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 for 5 minutes.

6. For antigen retrieval: immerse the slide in Tris-EDTA buffer, pH 9.0, 0.05% Tween- 20*, and incubate at 95 degrees C in water bath for 30 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.

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 for 10 minutes.

16. Stain in hematoxylin for 5 minutes.

17. Wash in water for 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.