

NBP1-79075 Protocol

Immunohistochemistry-Paraffin Protocol for Complement C4d Antibody (NBP1-79075)

IHC-P protocol (NBP1-79075):

1. Deparaffinize the section in 3 changes of xylene, 10 minutes each.
2. Wash the section in 96%, 80% and 70% benzyl alcohol for 10 minutes each.
3. Rinse in distilled water, 2 x 5 minutes.
4. Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H₂O₂) for 10 minutes.
5. Wash in distilled water, 2 x 5 minutes.
6. For antigen retrieval: immerse the slide in the citrate buffer, pH 6.0, 0.05% Tween-20*, and incubate in microwave (600W) for 10 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 citrate buffer, pH 6.0) for 20 minutes.
8. Rinse in distilled water, 2 x 5 minutes.
9. Wash in 0.05 M Tris-HCl, pH 7.6 buffer supplemented with 0.2% of Tween-20 (buffer A), 2 x 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 3 x 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 3 x 5 minutes with buffer A.
14. Apply the chromogen (DAB), 10 minutes.
15. Wash in water, 2 x 5 minutes.
16. Rinse in solution containing 150mM CuSO₄.5H₂O and 20mM NaCl.
17. Wash in distilled water, 1 x 2 minutes.
18. Stain in hematoxylin for 5 minutes.
19. Wash in distilled water, 3 x 2 minutes.
20. Rinse in 37mM ammonium hydroxide solution.
21. Wash in distilled water, 1 x 2 minutes.
22. Mount the slide for observation.