

## NBP1-79004 Protocol

### Immunohistochemistry-Paraffin protocol for Progesterone R/NR3C3 Antibody (NBP1-79004)

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[https://www.novusbio.com/products/progesterone-r-nr3c3-antibody-p21-s\\_nbp1-79004](https://www.novusbio.com/products/progesterone-r-nr3c3-antibody-p21-s_nbp1-79004)

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 (H<sub>2</sub>O<sub>2</sub>) 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 water bath at 90-C 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 - 400 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. Rinse 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.