

## NBP1-79009 Protocol

### Immunohistochemistry-Paraffin protocol for LMP1 Antibody (NBP1-79009)

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[https://www.novusbio.com/products/lmp1-antibody-s20-d\\_nbp1-79009](https://www.novusbio.com/products/lmp1-antibody-s20-d_nbp1-79009)

1. Deparaffinize the section in 3 changes of xylene, 5 minutes each.
2. Wash the section in 96%, 80% and 70% ethyl 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 for 5 minutes.
6. For antigen retrieval: immerse the slide in the citrate buffer, pH 6.0, 0.05% Tween-20\*, and incubate in water bath at 96C for 40 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.
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% ethyl alcohol for 5 minutes each.
19. Wash the section in 2 changes of xylene for 2 minutes each.
20. Mount the slide for observation