

## Immunohistochemistry Protocol (Frozen)

1. Wash sections in 1X TBS two times for 5 minutes.
2. Incubate for 10 minutes at room temperature in methanol/peroxidase.
3. Wash sections in 1X TBS two times for 5 minutes.
4. Block each section with 100–400  $\mu$ l of 1X TBS/0.3% Triton X-100/5% Normal Goat Serum for 1 hour at room temperature.
5. Remove blocking solution and add 100–400  $\mu$ l of primary antibody diluted in 1X TBS/0.3% Triton X-100/5% Normal Goat Serum to each section.
6. Incubate overnight at 4°C.
7. Return to room temperature.
8. Remove antibody solution and wash sections in 1X TBS three times for 5 minutes each.
9. Incubate in a humidified chamber for 30 minutes at room temperature.
10. Wash sections three times with 1X TBS for 5 minutes each.
11. Prepare an appropriate volume of substrate solution prior to use by mixing one drop of Liquid DAB plus chromogen immediately with 1 ml of substrate buffer. Apply the substrate carefully and incubate for 5-10 minutes till a brown color develops.
12. Immerse slides in dH<sub>2</sub>O.
13. To stain nuclei, immerse slides in a bath of hematoxylin for 3 minutes.
14. Rinse slides gently with distilled water.
15. Transfer slides into a 1% HCl, 99% ethanol solution for 10 seconds; transfer to distilled water immediately.
16. Wash sections in dH<sub>2</sub>O two times for 5 minutes each.
17. Dehydrate sections: Incubate sections in 95% ethanol two times for 10 seconds each. Repeat the incubation process in 100% ethanol. Repeat again in xylene.
18. Mount sections with coverslips.