Immunohistochemistry - Frozen Protocol

1. Wash sections in 1X TBS two times for 5 min.
2. Incubate for 10 min at room temperature in methanol/peroxidase.
3. Wash sections in 1X TBS two times for 5 min.
4. Block each section with 100–400 µl 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 µl 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 min each.
9. Incubate in a humidified chamber for 30 min at room temperature.
10. Wash sections three times with 1X TBS for 5 min 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 buff er. Apply the substrate carefully and incubate for 5-10 minutes till a brown color develops.
12. Immerse slides in dH_2O.
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_2O two times for 5 min each.
17. Dehydrate sections: Incubate sections in 95% ethanol two times for 10 sec each. Repeat in 100% ethanol, incubating sections two times for 10 sec each. Repeat in xylene, incubating sections two times for 10 sec each.
18. Mount sections with coverslips.