Immunohistochemistry on paraffin-embedded Protocol

1. Deparaffinizing twice, 10 min each time.
2. Rehydration
3. Antigen retrieval and then natural cooling
4. Primary antibody incubation. Incubate slides with 3 % H_2O_2 solution (100μL) for 15 min to quench endogenous peroxidase activity. Rinse slides with PBS, 3 times and 5 min each time.
5. Draw 2 lines beside tissue section to keep the liquid from flowing away.
6. Block the sections with corresponding serum or BSA.
7. Incubate sections with primary antibody overnight at 4 ℃. Then rinse slides with PBS, 3 times and 5 min each time.
8. Apply peroxidase labeled secondary antibody and incubate for 30 min. Rinse slides with PBS.
9. DAB plus chromogen
10. Hematoxylin or DAPI for nuclei staining.
11. Dehydration and mounting. Dehydrate sections with graded ethanol, 75 % (1 min), 85 % (1 min), 90 % (1 min), 100 % (1 min), 100 % (1 min). Immerse slides in xylene for 5 min. Repeat once.
12. Signal Detection. Mount and cover the section for detection.

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.