Immunohistochemistry Protocol (Paraffin)

1. Deparaffinize twice, 10 minutes each time.
2. Rehydrate.
3. Retrieve the antigen and then cool naturally.
4. Incubate slides with 3% H_2O_2 solution (100 µl) for 15 minutes to quench endogenous peroxidase activity. Rinse slides with PBS for 3 times, 5 minutes each time.
5. Draw 2 lines beside the 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°C. Then rinse slides with PBS for 3 times,5 minutes each time.
8. Apply peroxidase-labeled secondary antibody and incubate for 30 minutes. 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 minute), 85 % (1 minute), 90 % (1 minute), 100 % (1 minute), 100 % (1 minute). Immerse slides in xylene for 5 minutes. Repeat once.
12. Signal Detection. Mount and cover the section for detection.