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.

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