# Immunohistochemistry

# Protocol for SP Detection

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**Deparaffinization**

1. Incubate slide at 60℃ for 60 minutes.
2. Deparaffinize in Xylene for 10 minutes and repeat one more times.
3. Hydrate in 100% alcohol for 5 minutes, in 95% alcohol for 5 minutes, in 85% alcohol for 5 minutes, in 75% alcohol for 5 minutes.
4. Dip into Distill Water for 5 minutes.
5. Dip into TBS (50 mM Tris, 100 mM NaCl, pH 7.6), leave for 5 minutes, and repeat two times.

**Antigen Retrieval**

6. Bring 500 - 2000 ml 10 mM citrate buffer (pH6.0) to the boil in a stainless steel pressure cooker.
7. Put the slide into staining rack and lower into pressure cooker ensuring the slide is well immersed in citrate buffer.
8. When the pressure indicator valve has risen after 3-4 minutes, incubate for 1 minute.
9. Cool the slide naturally to room temperature.
10. Dip into distilled water, leave for 5 minutes, and repeat two times.
11. Dip the slide in TBS for 5 minutes and repeat two times.
12. Immerse slides in 3% $H_2O_2$ ( in fresh methanol) for 15 minutes at room temperature.
13. Wash with distilled water two times, 5 minutes each time.
14. Wash with TBS (pH 7.6) two times, 5 minutes each time.

**Staining with Primary Antibody**

15. Place the slide into Blocking Solution (3% BSA in TBS) for 30 minutes.
16. Dilute primary antibody with 3% BSA in TBS. Cover the tissue section on the slide with diluted primary antibody (use 50 – 150 µl for each slide).
17. Incubate at 37℃for 30 minutes or at room temperature for 60 minutes (The optimal incubation time, incubation temperature, and antibody dilution should be determined by the individual laboratory).
18. Wash with TBS two times, 5 minutes each time.

**Staining with Secondary Antibody**

19. Incubate with 100-200µl biotinylated secondary antibody diluted in Blocking Solution. Incubate 30 minutes at 37℃
20. Wash with TBS for 3 times, 5 minutes each time.
21. Incubate with 100-200µl streptavidin- HRP and incubate 30 minutes at 37℃
22. Wash with TBS for 3 times, 5 minutes each time.
23. Add DAB solution and incubate 10 minutes(The reaction progress and the optimal time should be determine according to microscope);
24. Wash with distilled water for 2 times, 5 minutes each time.
25. Counterstain sections in hematoxylin if required,wash with distilled water.Immerse slides in 0.1% HCl-ethanol for 1-10 seconds, wash with distilled water .
26. Dehydrate through 95% ethanol for 1 minute, 100% ethanol for 2×3min, Xylene for 2×3min, and coverslip with mounting medium.