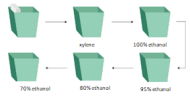
A Complete IHC Protocol

1. Deparaffinization and Rehydration



Wash slides with specific reagents in the following order:

- Xylene, two times, 15 min each.
- 100% ethanol, once, 2 min.
- 95% ethanol, once, 2 min.
- 80% ethanol, once, 2 min.
- 70% ethanol, once, 2 min.

Rinse sections with distilled water two times for 2 min each.

2. Blocking Endogenous Enzymes (Recommended)

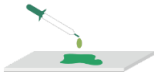
Block the endogenous peroxidase activity at room temperature by a 5-10 min incubation in the final developmental 3% H_2O_2 in distilled water or PBS (pH 7.4).
Rinse sections with PBS for 5 min.

3. Antigen Retrieval

Pour 1500 ml of Tris-EDTA buffer in pressure cooker, program to run for 3 min until the air valve rises.
Let it cool down to room temperature for 10-20 min.

Rinse sections with PBS for 5 min.

4. Serum Blocking



Apply the blocking serum, incubate for 30 min at room temperature, and throw off residual fluid which does not need to be washed.

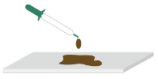
5. Primary Antibody Incubation

Apply the primary antibody at 37 °C for 60 min or 4 °C for overnight.
Rinse sections twice for 5 min each.

6. Secondary Antibody Incubation

Incubate array slide with a HRP-conjugated secondary antibody at 37 °C for 30 min.
Rinse sections twice for 5min each.

7. DAB Staining



Proceed with chromogen of final developmental DAB or use DAB Kit (Control the degree of staining with regular microscopy).
Wash sections in distilled water.

8. Counterstain

Counterstain slides by immersing sides in Hematoxylin for 4 min.

Realize the dehydration and transparency of slides.

9. Mounting

Mount slides.