Immunohistochemistry (IHC) Protocol-Staining Protocol

1. Deparaffinize:

Wash slides with specific reagents in the following order:

xylene, two times, 5 min each.

100% ethanol, two times, 5min each

95% ethanol, two times, 5 min each

80% ethanol, once, 5 min

70% ethanol, once, 5 min

50% ethanol, once, 5min

dH2O, two times, 5 min each

2. (Recommended) Block the endogenous peroxidase activity at room temperature by a 5~10 min incubation in the final developmental 3% H2O2 in distilled water or PBS (pH 7.4).

3. Rinse sections with PBS for 5 min.

4. Antigen retrieval.

5. Rinse sections with PBS for 5 min.

6. Apply the blocking antibody (normal goat serum), incubate for 30 min at room temperature, and throw off residual fluid (**don't wash**.).

7. Apply the primary antibody 60 min at RT or 4°C for overnight

8. Rinse sections 3 times for 5 min each.

9. Incubate array slide with a HRP-conjugated secondary antibody at 37°C for 40 min.

10. Rinse sections 3 times for 5min each.

11. Proceed with chromogen of final developmental DAB or use DAB Kit (Control the degree of staining with regular microscopy).

12. Wash sections in distilled water.

13. Stain and differentiate slides in hematoxylin.

14. Dehydration and transparency of slides.

15. Mount slides.