

## **Immunohistochemistry Method**

Deparaffinize sections and rehydrate using PBS.

Pre-treat the sample with **one** of the following procedures:

1. No treatment at all.
- \*2. Place sample in 1X citrate buffer (pH 6.0) and microwave at 750W for 20 minutes, cool sample subsequently.
3. Place sample in 1X Tris/EDTA buffer (pH 9.0) and microwave at 750W for 20 minutes, cool sample subsequently.
4. Place sample in HCl (2N) (pH 0.6~0.9) at room temperature for 10~20 minutes.
5. Place sample in 0.1% trypsin\* and shake for 25 minutes at 37°C.

### **Step-by-step procedure:**

1. Incubate sections in 3% H<sub>2</sub>O<sub>2</sub> in 1X PBS at room temperature for 10 minutes and then wash the sections again.
2. Incubate sections in blocking solution for 10 minutes.
3. Add primary antibodies (diluted in blocking solution) and incubate the sections overnight at 4°C, wash sample with 1X PBS afterwards.
4. Incubate sections with labeled polymer for 30 min followed by washing the sections with PBS.
5. Application of substrate solution (DAB or other suitable peroxidase substrate). Wash sample thoroughly under running tap water.
6. Counter stain the samples in Mayer's hematoxylin.
7. Dehydrate and mount samples.

### **Reagents:**

- Antibody Diluent (DAKO S3022)
- Immunodetection Kit- EnVision Detection Kit, Peroxidase/DAB, Rabbit/Mouse (DAKO K5007)
- Citrate buffer, pH 6.0: 10 mM sodium citrate buffer
- 1X Tris/EDTA, pH 9.0: 10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20
- HCl solution (2N), pH 0.6~0.9: prepared in distilled water

\* Method employed by Abnova.