

Immunohistochemistry (without pretreatment)

1. **Deparaffinization**
2. **Xylen substitution for Ethanol**
3. **Inactivation of endogenous peroxidases: 0.3% H₂O₂ in Methanol, room temp. 30 min.**
4. **Hydration:**
80% Ethanol → 70% Ethanol → 60% Ethanol → rinse by running water, 1 min.
5. **Rinse by TBS-T**
6. **Blocking: 5% normal serum (# 17301 Goat Whole Serum), room temp. 30 min.**
7. **Wash by TBS-T**
8. **Primary antibody incubation: 4°C overnight**
9. **Wash by TBS-T, 5 min. x 3**
10. **Secondary antibody incubation: room temp. 30 min.**
Anti-Rabbit IgG (H+L) Goat IgG-Biotin (IBL #17542), 10µg/mL
or
Anti-Mouse IgG (H+L) Goat IgG-Biotin (IBL #17641), 10µg/mL
11. **Wash by TBS-T, 5 min. x 3**
12. **Staining system: room temp. 30 min.**
(Vectastatin ABC Kit, PEROXIDASE STANDARD PK-4000)
13. **Wash by TBS-T, 5 min. x 3**
14. **Chromogenic reaction: room temp. 1-10 min.**
(DAB, " DOJINDO 349-00903" 30 mg, 30 % H₂O₂ 25 µL/50 mM Tris-HCl, pH 7.6, 150 mL)
15. **Rinse by running water, 3 min.**
16. **Counter staining**