

Immunohistochemistry (Autoclave 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. **Autoclave treatment*: 110 °C, 10 min (10 mM Citrate buffer, pH 6.0)**
6. **Cool down (leave it lay).**
7. **Rinse by running water, 3 min.**
8. **Rinse by TBS-T**
9. **Blocking: 5% normal serum (# 17301 Goat Whole Serum), room temp. 30 min.**
10. **Wash by TBS-T**
11. **Primary antibody incubation: 4°C overnight**
12. **Wash by TBS-T, 5 min. x 3**
13. **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
14. **Wash by TBS-T, 5 min. x 3**
15. **Staining system: room temp. 30 min.**
(Vectastatin ABC Kit, PEROXIDASE STANDARD PK-4000)
16. **Wash by TBS-T, 5 min. x 3**
17. **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)
18. **Rinse by running water, 3 min.**
19. **Counter staining**

*Autoclave treatment

- 1) Put 500mL of buffer into a 500mL beaker, and then soak the whole basket with tissue sections inside in the beaker.
- 2) Heat for 10 minutes (110 °C) by autovlave.

Preparation of 10 mM Citrate buffer, pH 6.0

- 1) Solve 2.1 g of citric acid (C₃H₄(OH)(COOH)₃/H₂O=210.14) in 900 mL deionized water.
- 2) Adjust the pH to 6.0 with sodium hydroxide solution (add about 13 mL as 2M-NaOH).
- 3) Fill deionized water to a volume of 1,000 mL.