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# 7TM Immunohistochemistry Protocol

**This protocol is designed for the staining of formalin-fixed, paraffin-embedded (FFPE) tissue sections.**

## **1. Buffers and Reagents**

Use double distilled water for buffer preparation or water with the same grade of purity.

- Citrate buffer:  $C_6H_8O_7 \times H_2O$ : 1.8 mM,  $C_6H_5Na_3O_7 \times H_2O$ : 8.7 mM, pH 6.0
- PBS: Phosphate Buffered Saline (NaCl: 150 mM,  $KH_2PO_4$ : 50 mM)
- PBS/BSA: PBS with 1% BSA (bovine serum albumin)

## **2. Deparaffinization, Rehydration and Antigen Unmasking**

1. Wash sections with xylene for 20 min. Aspirate xylene. Repeat 3-times.
2. Wash sections with 100% ethanol for 20 min. Aspirate ethanol. Repeat 3-times.
3. Blocking of endogenous peroxidase: Incubation with 0.15%  $H_2O_2$  in methanol for 45 min.
4. Wash sections with 95% ethanol for 2 min. Aspirate ethanol. Repeat 2-times.
5. Wash sections with 80% ethanol for 2 min. Aspirate ethanol.
6. Wash sections with 70% ethanol for 2 min. Aspirate ethanol.
7. Wash sections with water for 5 min. Aspirate water. Repeat 2-times.
8. Use a microwave for the following boiling/cooling cycles in citrate buffer:
  - boil for 8 min
  - cool at room temperature for 4 min
  - boil for 4 min
  - cool at room temperature for 4 min

- boil for 4 min
  - cool at room temperature for 20 min
9. Aspirate citrate buffer. Incubate 5 min in PBS/BSA.

### **3. Staining and Mounting**

1. We recommend the use of a Shandon™ Sequenza™ staining rack for the following steps.
2. Wash with 1.5 ml PBS/BSA for 5 min.
3. Incubate sections with 300 µl 7TM Premium IHC-Grade Antibodies at a dilution of 1:100 in PBS/BSA for 1-2 hours at room temperature or at 4 °C overnight (we recommend the incubation overnight).
4. Wash with 1.5 ml PBS/BSA for 5 min.
5. Incubate sections with 150 µl biotinylated anti-rabbit-IgG of your choice for 20 min.
6. Wash with 1.5 ml PBS/BSA for 5 min.
7. Incubate sections with 150 µl peroxidase-conjugated streptavidin of your choice for 20 min.
8. Wash with 1.5 ml PBS/BSA for 5 min.
9. Incubate in 3-amino-9-ethylcarbazole (AEC) solution of your choice for 15 min.  
Repeat 2-times.
10. Wash with 1.5 ml water for 5 min.
11. Wash with water for 1 min. Repeat 2-times.
12. Incubate sections with hematoxylin solution of your choice for 5 min.
13. Wash with water for 2.5 min
14. Dip sections in 250 ml water containing 1.4 ml concentrated ammonia.  
Repeat 5-times.
15. Wash with water for 2.5 min.
16. Mount sections by using an aqueous mounting media on microscope slides.