



## General IHC with Epitope Retrieval Protocol

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### Description:

Formalin or other aldehyde fixation forms protein cross-links that mask the antigenic sites in tissue specimens, leading to weak or false negative staining for immunohistochemical detection of certain proteins. Sodium citrate treatments breaks the protein cross-links, unmasking the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, enhancing staining intensity of antibodies

### Procedure:

1. Deparaffinize sections in 3 changes of xylene, 5 minutes each
2. Hydrate in 2 changes of 100% ethanol for 3 minutes each, 95% and 80% ethanol for 1 minute each.
3. Rinse the sections in distilled water followed by 0.1 M PBS.
4. Quench endogenous peroxidase for 10 min with 3% H<sub>2</sub>O<sub>2</sub> in 0.1 M PBS and wash 3 times with distilled water, 2 minutes each.
5. Retrieve epitopes by incubating the sections at 98-100° C in a microwave in 0.01 M citrate buffer (pH 6.0) for 10 minutes. Cool down in room temperature for 30-60 min.
6. Rinse the sections in 0.1 M PBS for 3 × 2 min.
7. Block the sections with 10% normal goat serum in 0.1 M PBS at room temperature for 30 min.
8. Incubate the sections with primary antibody at appropriate dilution in 0.1 M PBS (2-4µg/ml) overnight at 4 ° C.
9. Rinse the sections in 0.1 M PBS for 3 × 5 min.
10. Incubate the sections with biotinylated goat anti-rabbit IgG (SPN-9001, Vector, 1/200 in 0.1M PBS, 37° C, 20 min)
11. Rinse the sections in 0.1 M PBS for 3 × 5 min.
12. Incubate the sections with HRP-conjugated streptavidin (SPN-9001, Vector, 1/200 in 0.1 M PBS, 37 ° C, 20 min)
13. Rinse the sections with 0.1 M PBS for 3 × 5 min followed by 50 mM Tris-HCl buffer (pH 7.6).
14. Incubate with a peroxidase substrate solution.
15. Rinse the sections in distilled water.
16. Counterstain for 10-30 seconds with a hematoxylin counterstaining solution.
17. Rinse the sections with tap water.



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18. Dehydrate through 70-80-90-95-100-100% of ethanols, each 3 minutes.

19. Clear in xylene for 2×3 min.

20. Coverslip with mounting medium.

### Solutions and Reagents:

#### **1: 0.01 M Citrate Buffer (pH 6.0)**

Tris-sodium citrate ----- 2.94 g

Distilled water ----- 1000 ml

Mix to dissolve. Adjust pH to 6.0 with 1N HCl.

Note: this buffer is commonly used and works perfectly with many antibodies. It gives very nice intense staining with very low background.

#### **2: 0.1 M PBS**

Na <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O	27.2g	54.5g
NaH <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O	2.8g	5.6g
NaCl	9g	18g
Distilled water	1000ml	2000ml
pH	7.4	7.4

#### **3: 50 mM Tris-HCl Buffer**

Tris	6g	12g
5N HCl	5-7ml	10-14ml
pH	7.6	7.6
Distilled water	1000ml	2000ml

#### **4: Peroxydase Substrate Solution**

50 mM Tris-HCl (pH 7.6)	49 ml
1% DAB	1 ml
30% H <sub>2</sub> O <sub>2</sub>	7 µl

#### **5: Hematoxylin Counterstaining Solution**

Hematoxylin	1g
Distilled water	1000ml
NaIO <sub>3</sub>	0.2g
KAl(SO <sub>4</sub> ) <sub>2</sub> •12H <sub>2</sub> O	50g
Citric acid	1g
Trichloroacetaldehyde	50g

After hematoxylin is completely dissolved in distilled water, add NaIO<sub>3</sub> and KAl (SO<sub>4</sub>)<sub>2</sub>.12H<sub>2</sub>O, stir until thoroughly dissolved. Then add citric acid and trichloroacetaldehyde stir until thoroughly dissolved.