

Citrate Buffer Epitope Retrieval Method

Description: Formalin or other aldehyde fixation forms protein cross-links that mask the antigenic sites in tissue specimens, thereby giving weak or false negative staining for immunohistochemical detection of certain proteins. The sodium citrate based solution is designed to break the protein cross-links, therefore unmask the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, thus enhancing staining intensity of antibodies.

Solutions and Reagents:

Citrate Buffer (10mM Sodium Citrate Buffer, 0.05% Tween 20, pH 6.0):

Tri-sodium citrate ----- 2.94 g

Distilled water ----- 1000 ml

Mix to dissolve. Adjust pH to 6.0 with 1N HCl and then add 0.5 ml of Tween 20 and mix well.

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

Procedure:

1. Deparaffinize sections in 2 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. Then rinse in distilled water.
3. Pre-heat steamer or water bath with staining dish containing citrate buffer until temperature reaches 95-100 °C.
4. Immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20-40 minutes (optimal incubation time should be determined by user).
5. Turn off steamer or water bath and remove the staining dish to room temperature and allow the slides to cool for 20 minutes.
6. Rinse sections in washing buffer for 2x2 min.
7. Block sections with normal serum blocking solution for 30 minutes.

8. Perform avidin/biotin blocking if necessary.
9. Incubate sections with primary antibody at appropriate dilution in primary antibody dilution buffer for 1 hour at room temperature or overnight at 4 °C.
10. Rinse sections with washing buffer for 2x2 min.
11. Block sections with peroxidase blocking solution for 10 minutes.
12. Rinse with washing buffer for 3x2 min.
13. Proceed to standard immunohistochemistry staining procedure.

Note: Microwave or pressure cooker can be used as alternative heating source to replace steamer or water bath.