



## **Immunohistochemistry Protocol for Paraffin Sections –Avidin-Biotin Complex Detection-**

### **Solutions and Reagents**

*Note: Prepare solutions with Milli-Q or equivalently purified water. Be aware that azide will interfere with the HRP enzyme reaction and should not be added to solutions.*

- Xylene
  
- Ethanol (100 % and 95 %)
  
- Distilled H<sub>2</sub>O (dH<sub>2</sub>O)
  
- Hematoxylin
  
- 10X Phosphate Buffered Saline (PBS): 0.58 M sodium phosphate dibasic (Na<sub>2</sub>HPO<sub>4</sub>), 0.17 M sodium phosphate monobasic (NaH<sub>2</sub>PO<sub>4</sub>), 0.68 M NaCl. To prepare 1 liter of 10X PBS, combine 82.33 g Na<sub>2</sub>HPO<sub>4</sub>, 23.45 g NaH<sub>2</sub>PO<sub>4</sub> H<sub>2</sub>O and 40 g NaCl. Adjust pH to 7.4.
  
- 10 mM Sodium Citrate Buffer: To prepare 1 liter, add 2.94 g sodium citrate to 1 liter dH<sub>2</sub>O. Adjust pH to 6.0.
  
- 1 % Hydrogen Peroxide: To prepare, add 10 ml 30 % H<sub>2</sub>O<sub>2</sub> to 290 ml dH<sub>2</sub>O.
  
- Blocking Solution: 5 % horse serum or goat serum in PBS
  
- ABC Reagent: (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
  
- DAB Reagent: Add 7 µl of 30 % hydrogen peroxide to 10 ml dH<sub>2</sub>O; add this mixture to 10 ml of 1 mg/ml DAB (diaminobenzidine tetrahydrochloride) in PBS, filtrate.

## Protocol

1. Deparaffinize/hydrate sections:
  - a. Incubate sections in three washes of xylene for 5 minutes each.
  - b. Incubate sections in two washes of 100 % ethanol for 10 minutes each.
  - c. Incubate sections in two washes of 95 % ethanol for 10 minutes each.
2. Wash sections twice in dH<sub>2</sub>O for 5 minutes each.
3. Wash sections in PBS for 5 minutes.
4. For antigen unmasking, heat sections in 10 mM sodium citrate buffer (pH 6.0) for 1 minute at full power followed by 9 minutes at medium power. (Keep slides fully immersed in buffer and maintain temperature at or just below boiling.) Cool slides for 20 minutes after antigen unmasking.
5. Wash sections in dH<sub>2</sub>O three times for 5 minutes each.
6. Incubate sections in 1 % hydrogen peroxide for 10 minutes.
7. Wash sections in dH<sub>2</sub>O three times for 5 minutes each.
8. Wash section in PBS for 5 minutes.
9. Block each section with 100 µl blocking solution for 1 hour at room temperature.
10. Remove blocking solution and add 10-40 µl diluted primary antibody to each section. (Dilute antibody in blocking solution.) Incubate overnight at 4°C.
11. Remove antibody solution and wash sections in PBS three times for 5 minutes each.
12. Add 10-40 µl secondary antibody, diluted in blocking solution, to each section. Incubate 30 minutes at room temperature.
13. If using ABC avidin-biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
14. Remove secondary antibody solution and wash sections three times with PBS for 5 minutes each.

15. Add 10-40  $\mu$ l ABC reagent to each section and incubate for 30 minutes at room temperature.
16. Remove ABC reagent and wash sections three times in PBS for 5 minutes each.
17. Add 10-40  $\mu$ l DAB reagent to each section and monitor staining closely.
18. As soon as the section turns brown, immerse slides in dH<sub>2</sub>O.
19. If desired, counterstain sections in hematoxylin or methylgreen for 10 seconds.
20. Wash sections in dH<sub>2</sub>O two times for 5 minutes each.
21. Dehydrate sections:
  - a. Incubate sections in 95 % ethanol two times for 10 seconds each.
  - b. Repeat in 100 % ethanol, incubating sections two times for 10 seconds each.
  - c. Repeat in xylene, incubating sections two times for 10 seconds each.
22. Mount coverslips with mounting media.