Detection of Isolectin B4 in Formalin-Fixed, Paraffin-Embedded Mouse and Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1mM CaCl$_2$, MgCl$_2$, and MnCl$_2$
1X Citrate Buffer
DAB Chromogen
Hematoxylin

Lectin: Isolectin B$_4$ (BSI-B$_4$), Peroxidase Conjugated (Griffonia simplicifolia)
Sigma-Aldrich, Inc
St Louis, MO 63178
1-800-558-9160
www.sigmaaldrich.com
Catalog # L5391

Staining Procedure

Positive Control Tissue: Brain
Stain Localization: Cytoplasmic (microglial cells)

1. Deparaffinize and hydrate slides through the following solutions:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Repetitions</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylene</td>
<td>2 times</td>
<td>5 minutes</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>2 times</td>
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2. Heat-Induced Epitope Retrieval Using The Decloaker
Add 500 ml of distilled water to the pan inside the decloaker.
Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
(Insert blank slides into any empty slots in the rack to ensure even heating of slides)
Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure ________*
Depressurize for 10 minutes.
Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides ________*
Rinse the slides in 2 changes of distilled water for 3 minutes each time.

3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

4. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
6. Incubate the slides in a 1mM CaCl$_2$, MgCl$_2$, and MnCl$_2$ solution for 30 minutes at room temperature.

7. Incubate the slides in 1% BSA diluent for 10 minutes at room temperature.

8. Quickly rinse the slides in 1mM CaCl$_2$, MgCl$_2$, and MnCl$_2$.

9. Apply the IB$_4$-HRP lectin at a 1:75 dilution and incubate overnight at 4°C.
   (Use the 1mM CaCl$_2$, MgCl$_2$, and MnCl$_2$ solution as the diluent for the lectin reagent.)

*************Next Day*************

10. Bring the slides up to room temperature in 1X wash buffer for at least 15 minutes.

14. Apply the DAB chromogen and incubate in the dark for 6 minutes at room temperature.
   (Add 1 drop of DAB per ml of substrate)

   Lot #________________ Exp. Date________________ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X wash buffer until they turn blue.

19. Dehydrate through the following solutions:

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20. Coverslip

Updated 05/14/04