

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 CaCl₂, MgCl₂, and MnCl₂](#)

[1X Citrate Buffer](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Lectin: Isolectin B₄ (BSI-B₄), Peroxidase Conjugated (*Griffonia simplicifolia*)

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Staining Procedure

Positive Control Tissue: Brain

Stain Localization: Cytoplasmic (microglial cells)

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

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 CaCl₂, MgCl₂, and MnCl₂ 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 CaCl₂, MgCl₂, and MnCl₂.
9. Apply the IB₄-HRP lectin at a 1:75 dilution and incubate overnight at 4°C.
(Use the 1mM CaCl₂, MgCl₂, and MnCl₂ 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:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

Updated 05/14/04