

Detection of Iba1 in Formalin Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)
[3% Hydrogen Peroxide](#)
[1% BSA Diluent](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Staining Kit: Vectastain Elite ABC Kit (Rabbit IgG)

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6101

Note: This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary Antibody: Rabbit Anti-Iba1 Antibody

Wako Chemicals USA, Inc
Richmond, VA 23237
www.wakousa.com
1-804-271-7677
Catalog #019-19741

Negative Control Serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

Staining Procedure

Positive Control Tissue: Brain

Stain Localization: Cytoplasmic and nuclear

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. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.
3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

Vectastain Rabbit Elite Staining Kit

Exp Date _____ New Kit: yes / no

4. Apply the block from the Rabbit Elite Kit, and incubate for 20 minutes at room temperature.

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

5. Avidin / Biotin Blocking Kit

Lot # _____ Exp. Date _____ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BUFFER.

6. Apply primary antibody at a 1:100 dilution, and incubate for 1 hour at room temperature.

Lot # _____ Exp Date _____

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:100 dilution from this normalized serum, and apply to the slides.

Incubate for 1 hour at room temperature.

Lot # _____ Date Reconstituted _____

7. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
8. Apply the secondary antibody from Rabbit Elite Kit, and incubate for 30 minutes at room temperature.
9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

10. Apply the label complex from the Rabbit Elite Kit, and incubate for 30 minutes at room temperature.
(Prepare at least 30 minutes prior to use.)
11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
12. Apply the DAB chromagen, 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
13. Rinse the slides in tap water 3 minutes.
14. Counterstain with Harris Hematoxylin for 20 seconds.
15. Rinse the slides in tap water until water is clear.
16. Gently agitate slides in 1X Wash Buffer until they turn blue.
17. 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

18. Coverslip

Updated 09/05/07