ANTI- IBA1 LABELING KIT

OVERVIEW

Product Name: Rabbit monoclonal [EPR16589] to Iba1 (ab178847)

Description: Tissue labeling kit for Iba1

Host Species: Rabbit IgG

Size: 1 mm thick coronal section of mouse brain

Storage: Store antibody at +4˚C short term (1-2 weeks). Store at -20˚C or -80˚C indefinitely. Avoid freeze / thaw cycle. Store Visikol HISTO-1 and HISTO-2 at room temperature and Visikol HISTO buffers at +4˚C.

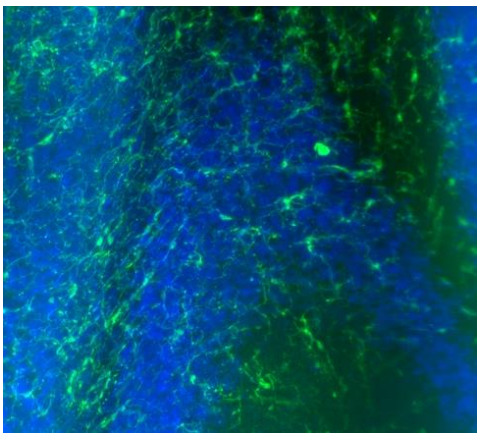

Fig 1. Mouse Brain Section Labeled with Iba1 (Green, ab178847 1:100) and DAPI (Blue)

Materials In Kit

- Anti-Iba1 (ab178847)
- Visikol® HISTO-1™ – 30 mL
- Visikol® HISTO-2™ – 30 mL
- Visikol® HISTO™ Penetration Buffer – 30 mL
- Visikol® HISTO™ Washing Buffer 10X – 70 mL
- Visikol® HISTO™ Blocking Buffer – 30 mL
- Visikol® HISTO™ Antibody Buffer – 30 mL
- Visikol® HISTO™ Permeabilization Buffer – 30 mL

Materials Required

- DAPI
- Methanol (ethanol if FP labeling)
- PBS
- PBS with 1% Triton™ X-100
- DMSO

PROTOCOL

Tissue Processing

1. Fix tissue in your chosen fixative. After fixing for 24 hours at 4˚C, remove and transfer to PBS with 0.05% sodium azide for indefinite storage at 4˚C.
2. Wash tissue in at least 5 mL PBS solution for at least 30 minutes before further procedures to remove traces of fixative.

If tissues have been cryopreserved, wash an additional 3-5 times with 5 mL PBS for at least 30 minutes to ensure complete removal of the mounting media.

Permeabilization

1. Incubate tissue section in Permeabilization Buffer at room temperature for 1 hour with gentle shaking.

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2. Wash tissue in 5 mL PBS 3X for at least 30 minutes each to remove excess Permeabilization Buffer before continuing on.
3. Wash tissue in increasing concentrations of methanol at room temperature to dehydrate the tissue. First wash in 5 mL PBS, then 5 mL of 50% methanol in PBS, 80% methanol in DI H_2O, and finally 100% methanol for 30 minutes each with gentle shaking. Samples can be stored in methanol (preferably at 4ºC) indefinitely before proceeding with the next step.
4. Wash samples at room temperature with 5 mL 20% DMSO/methanol for 30 minutes.
5. Wash tissue in decreasing concentrations of methanol at room temperature to rehydrate the tissue. First wash in 80% methanol in DI H_2O, then 50% methanol in PBS, and finally 100% PBS for 30 miuntes each with gentle shaking.
6. Wash samples at room temperature with 5 mL PBS/1% Triton™ X-100 for 30 minutes.
7. Incubate samples at room temperature in 5 mL Penetration Buffer for 1 hour with gentle shaking.
8. Block samples in 5 mL Blocking Buffer at 37˚C with gentle shaking for at least 2 hours up to overnight.
9. Incubate samples in a primary antibody dilution prepared in 2 mL Antibody Buffer and incubate at 37˚C with gentle shaking for 4 hours. For ab178847, use a dilution of 1:100. Incubate for 48 hours. For more uniform labeling, incubate in a fresh dilution of antibody after 24 hours.
10. Be sure to dilute the 10X Washing Buffer to a 1X working concentration before using.
11. Wash samples in 5 mL 1X Washing Buffer (5 times, 30 minutes each, at 37˚C, with gentle shaking).
12. Add desired secondary to 2 mL of antibody buffer. For a Goat Anti-Rabbit IgG H&L AlexaFluor488 (ab150077), use a dilution of 1:400 for a final concentration of ~5 ug/mL. To this solution, also add a nucelar counterstain of choice. We typically recommend DAPI at a dilution of 1:1000.
13. Incubate tissue in secondary antibody at 37˚C with gentle shaking. Incubate samples for 24 hours.
14. Wash in 5 mL 1X Washing Buffer (5 times, 30 minutes each time, at 37˚C, with gentle shaking). Transfer to 5 mL PBS. Samples may be kept in this solution indefinitely at 4˚C before proceeding with further steps.

Tissue Clearing

1. Wash tissue at room temperature with 5 mL PBS, then 50% methanol for 30 minutes, 80% methanol, and finally 100% methanol for 1 hour with gentle shaking.
2. Remove methanol and wpie excess with KimWipe.
3. Add 5 mL Visikol HISTO-1 and let tissue equilibrate with the solution for at least 2 hours. Then add 5 mL HISTO-2 and wait until cleared (usually <2 hours) before proceeding directly to imaging.

Imaging Guidance

Warning! Do not treat specimens with other media after clearing. For optimal results, imaging should be performed directly in Visikol HISTO reagents. Visikol HISTO solutions contain anti-fade agents, and specimens cleared with Visikol HISTO do not need to be mounted in an additional anti-fade media. Other solutions (particularly aqueous media) will cloud the tissue or completely reverse the clearing, interfering with 3D volume imaging. Once the cleared tissues have been successfully imaged, see below for additional information on reversing the clearing to perform downstream assays.

After 3D imaging, if desired, additional histological or biochemical assays can be performed on Visikol HISTO treated tissue by reversing the clearing. This can be simply done by washing the tissue three times with 5 mL 100% ethanol at room temperature. The tissue can then be processed as any fixed tissue, for SDS-PAGE, Western Blotting, or embedding, sectioning, and staining with H&E, Nissl, IHC or other histological stains.

https://www.youtube.com/watch?v=oclD_jE4wm8

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