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Working

# UC Davis - Immunohistochemistry IBA1

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ABSTRACT

**Summary:**

Ionized calcium-binding adapter molecule 1 (IBA1) is specifically expressed in macrophages / microglia and is upregulated during the activation of these cells. Iba1 expression is up-regulated in microglia following nerve injury,[4] central nervous system ischemia, and several other brain diseases. Furthermore it has been found in athlesclerotic plaques and at sigts of vascular injury.

**Modified from**: IHC Methods and Materials VMTH - Anatomic Pathology, UC-Davis

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=257&docType=Protocol

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| xylene | | |
| ethanol | | |
| Hydrogen peroxide | | |
| Methanol | | |
| Target retrieval solution | S1699 | Dako |
| 0.1M Phosphate Buffered Saline pH 7.4 | | |
| Normal horse serum | | |
| Tween-20 | | |
| IBA Ab | 019-19741 | Wako |
| polymer based HRP | RC542H | Biocare Medical |
| NovaRed for peroxidase | SK-4800 | Vector Laboratories |
| Mayer's Hematoxylin | S3309 | Dako |
| coverslip | 2935-245 | Corning |

MATERIALS TEXT

**Reagent Preparation:**

**Reagent1:** PBS-Tween 20
Reagents and Materials:
0.1M Phosphate buffered saline (PBS), pH 7.4 100mL

Tween 20 20µL

Procedure:
Mix to dissolve.

**Reagent 2:** Antibody diluent/blocking solution
Reagents and Materials:
PBS-Tween 20 90mL
Normal Horse Serum (NHS) 10mL

Procedure:
Mix to dissolve.

**Note:**

**Dako** RRID:SCR_013530

**Wako** RRID:SCR_013651

**Biocare Medical** RRID: SCR-013549

**Vector Laboratories,** RRID:SCR_000821

**IBA Ab #019-19741,** Cite this, **(Wako Cat# 019-19741,** RRID:AB_839504**)**

**NovaRed for peroxidase #SK-4800,** Cite this, **(Vector Laboratories Cat# SK-4800,** RRID:AB_2336845**)**

SAFETY WARNINGS

**WARNING:**

*Formalin is, toxic, flammable and considered a carcinogen*

*Xylene, ethanol and methanol are all flammable and should be used in fume hood away from open flames or sparks*

*All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.*

BEFORE STARTING

***WARNING:***
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1 Immunohistochemistry was performed on four-micron thick, formalin-fixed, paraffin-embedded tissue sections, mounted on charged slides, and air-dried overnight at 37°C.

2 Sections were deparaffinized through xylene to 100% reagent alcohol, and then treated with 0.3% hydrogen peroxide in 100% methanol for 30 minutes.

3 Sections were rehydrated to deionized water through 95% and 70% reagent alcohols. Antigen retrieval was performed on sections for IBA-1 with heat induced epitope retrieval in a Black & Decker Steamer using Target Retrieval Solution for 30 minutes at 95ºC, followed by a 20 minute cool down.

4 After antigen retrieval, slides were rinsed in deionized water and placed in 0.1M Phosphate Buffered Saline, pH 7.4 (PBS).

5 Sections were blocked for 20 minutes with antibody diluent and primary antibodies were applied without rinsing and incubated for 1 hour.
a. All post-antigen retrieval incubations are in a humidity chamber at room temperature.

6 After primary incubation, samples are rinsed twice for three minutes with PBS-Tween 20 between each subsequent reagent application.

7 A single step, polymer based HRP (BioCare Medical, RC542H) was applied for 30 minutes to label rabbit anti-IBA-1.

8 All labels were visualized with NovaRed for peroxidase (Vector SK-4800), per manufacturer's instructions.

9 Sections are counterstained in Mayer's Hematoxylin, air dried and coverslipped.