MMPC Immunohistochemistry

Ionized calcium-binding adapter molecule 1 (IBA1)

Version: 1
Modified from: IHC Methods and Materials VMTH - Anatomic Pathology, UC-Davis
Edited by: Dr. Rutkowsky, UC Davis

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.**

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
xylene		
ethanol		
Hydrogen peroxide		
Methanol		
Target retrieval solution	Dako	S1699
0.1M Phosphate Buffered Saline pH 7.4		
Normal horse serum		
Tween-20		
IBA Ab	Wako	019-19741
polymer based HRP	BioCare Medical	RC542H
NovaRed for peroxidase	Vector	SK-4800
Mayer's Hematoxylin	Dako	S3309
coverslip	Corning	2935-245

Protocol:

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.

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 95°C, 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.

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.