

Microglia (Iba1)

Concentrated Polyclonal Antibody
901-290-022619

BIOCARE
M E D I C A L

Catalog Number:	CP 290 A, B
Description:	0.1, 0.5 mL, conc.
Dilution:	1:200
Diluent:	Background Punisher

Intended Use:

For In Vitro Diagnostic Use

Microglia (Iba1) is a rabbit polyclonal antibody that is intended for laboratory use in the qualitative identification of Iba1 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

Studies have shown that Iba1 (ionizing calcium-binding adaptor molecule 1) is a novel protein that is specifically expressed in macrophages/microglia and is upregulated during the activation of these cells. Studies have shown cross-reactivity in human, mouse and rat tissues. Glial fibrillary acidic protein (GFAP) and microglia antibodies have been used as markers for axonal damage, reactive astrocytes and activated microglia, respectively. The Iba1 polyclonal antibody does not cross-react with neurons or astrocytes.

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Rabbit polyclonal

Species Reactivity: Human, mouse and rat

Clone: N/A

Isotype: N/A

Protein Concentration: Lot specific Ig concentration is not available.

Epitope/Antigen: Iba1

Cellular Localization: Cytoplasm of microglia and macrophages

Positive Tissue Control: Normal brain

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment Solution (recommended): Reveal or Diva, and Pepsin digestion

Pretreatment Protocol:

Heat Retrieval Method:

Preheat the retrieval solution to 80°C for 30 minutes and then place slides into the preheated solution if using Decloaking Chamber Pro or Decloaking Chamber Plus. If using Decloaking Chamber NxGen, place slides into the retrieval solution without preheating. Retrieve at 80°C for 30 minutes. Allow solution to cool for 20 minutes and then wash in distilled water.

Digestion Method:

Post digest after retrieval with Pepsin enzyme for 1 minute at RT.

Protocol Recommendations (intelliPATH FLX and manual use)

Cont'd:

Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a secondary-conjugated polymer.

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Notes:

1. This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 2 detection system. Use TBS for washing steps.
2. If you are using mouse or rat tissue use Rabbit-on-Rodent Polymer Kit (minimum cross-reactivity to mouse and rat tissues).
3. Optimal staining results are achieved when brain sections are cut at 7 microns.
4. Microglia staining may be improved with a post-DAB enhancement solution.

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (3)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (4)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

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Precautions Cont'd:

5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Ito D, *et al.* Microglia-specific localization of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res.* 1998 Jun 1;57(1):1-9.
2. Okere CO, Kaba H. Heterogenous immunohistochemical expression of microglia specific ionized calcium binding adaptor protein (Iba1) in the mouse olfactory bulb. *Brain Res.* 2000 Sep 15;877(1):85-90.
3. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
4. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.