Product Code:
MP-290-CR01 (0.1ml conc)  
MP-290-CR05 (0.5ml conc)

Product Description:
Microglia (Iba1)  
Concentrated Polyclonal Antibody  
Control Number: 901-290-030508  
ISO 9001:2000 CERTIFIED

Summary and Explanation:
Antigen detection, in tissues and cells, is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labelling the antigen with a primary antibody, a universal, affinity-purified, secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Recent studies have shown that Iba1 (ionizing calcium-binding adaptor molecule 1) is a novel protein that it 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.

Intended Use: For In Vitro Diagnostic Use

Source: Rabbit polyclonal

Species Reactivity: Human, mouse and rat

Clone: N/A

Isotype: N/A

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig Concentration.
Epitope/Antigen: Iba1

Cellular Localization: Cytoplasm of microglia and macrophages

Positive Control: Normal brain*

Normal Tissue: Brain

Abnormal Tissue: Glioma, astrocytoma

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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.
Instructions for Use:

**Endogenous Peroxidase Block**
Block all endogenous peroxidase activity by incubating the sections for 5 minutes with the MenaPath Peroxide Block. Rinse slides in water, and then rinse well in buffer.

**Pretreatment Protocol:**
Retrieve sections with MenaPath Access Supreme or Access Revelation Solution using the MenaPath Access Retrieval Unit at 80°C for 30 minutes followed by a wash in distilled water.

Alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 20 minutes then wash in distilled water.

**Digestion Method (Required):**
Digest with Pepsin enzyme for 1 minute at RT.

**Protein Block**
Incubate sections for 10-15 minutes at room temperature with the MenaPath Background Blocker with Casein

**Primary Antibody**
Dilute antibody 1:600-1:800 with MenaPath Universal Antibody Diluent. Incubate sections for 30 minutes at room temperature. Rinse slides x3 with buffer.

**Universal Probe**
Incubate sections for 10-20 minutes at room temperature with the MenaPath X-Cell Plus Universal Probe. Rinse slides x3 with buffer

**HRP Polymer**
Incubate sections for 10-25 minutes at room temperature with the MenaPath X-Cell Plus HRP Polymer. Rinse slides x3 in buffer.

**Chromogen**
Incubate sections for 5 minutes at room temperature with MenaPath X-Cell Plus Liquid Stable DAB. Rinse x3 with buffer.

**Counterstain:**
Incubate for 30-60 seconds with MenaPath Haematoxylin. Rinse with deionized water. Apply Bluing solution for 1 minute. Dehydrate, Clear and Mount
**Technical Note:** Use TBS buffer for wash steps.

* If you are using mouse or rat tissue use MenaPath Rabbit-on-Rodent Polymer Kit (minimum cross-reactivity to mouse and rat tissues)
* Optimal staining results are achieved when brain sections are cut at 7 microns.
**Microglia staining may be improved with a post-DAB enhancement solution.

**Performance Characteristics:** 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 MenaPath products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

**Quality Control:** Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about Tissue Controls.

**Precautions:** 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)

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 in contact with sensitive areas, wash with copious amounts of water.

Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.
**Troubleshooting:** Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Menarini’s Technical Service Helpline: on 01189 444130.

**Limitations and Warranty**
There are no warranties, expressed or implied, which extend beyond this description. Menarini is not liable for damages of any kind including: personal injury, or economic loss caused by this product.

**Reference:**