

Anti Iba1, Rabbit for Immunocytochemistry

<For Research Use Only>
Storage condition: Keep at -20°C

Antibodies against Macrophage/Microglia-specific Protein Iba1

Anti Iba1 polyclonal antibodies, Rabbit (Iba1: ionized calcium binding adapter molecule 1)

Calcium ions are known to be one of the most important signal mediators in all cells including central nervous system cells. Calcium ions exert their signaling activity through association with various calcium binding proteins, many of which are classified into a large protein family, the EF hand protein family.

Iba1 is a 17-kDa EF hand protein that is specifically expressed in macrophages/ microglia and is upregulated during the activation of these cells.

Wako has launched rabbit polyclonal antibodies which were raised against a synthetic peptide corresponding to the Iba1 carboxyl terminal sequence, which was conserved among human, rat and mouse Iba1 protein sequences. **These antibodies are specifically reactive to microglia/ macrophages, are appropriate for immuno-double staining of brain tissues and cell combination with monoclonal antibody to GFAP, which specifically reacts to astrocyte.**

Specificity :

Specific to microglia and macrophages, but not cross-reactive with neurons and astrocytes.

Reactive with human, mouse and rat Iba1.

Anti Iba1 polyclonal antibody, Rabbit, for Immunocytochemistry

Wako Cat. #019-19741 50 µg (100 µL)

-20 °C, D/I

Working Conc.: Immunocytochemistry 1 - 2 µg/mL

Anti Iba1 polyclonal antibody, Rabbit, for Western Blotting

Wako Cat. #016-20001 50 µg (100 µL)

-20 °C, D/I

Working Conc.: Westernblot 0.5 - 1 µg/mL

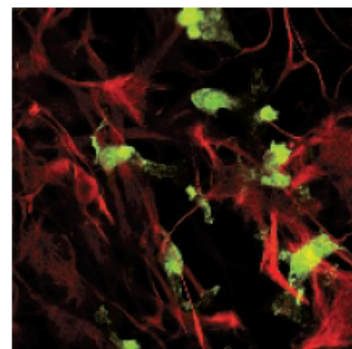


Figure 1

Immunocytochemistry of rat primary mixed glial cells.
Green: Iba1, which reacts to anti Iba1 antibody (Wako Cat. #019-19741)
Red: astrocyte, which reacts to anti GFAP monoclonal antibody
(Data was provided by Dept. of Neurochemistry, Institute of Neuroscience (Japan).)

[References]

- 1) Imai, Y., Iwata, I., Ito, D., Ohsawa, K. and Kohsaka, S.: *Biochem. Biophys. Res. Commun.*, **224**, 855 (1996).
- 2) Ito, D., Imai, Y., Ohsawa, K., Nakajima, K., Fukuuchi, Y. and Kohsaka, S.: *Brain Res. Mol. Brain Res.*, **57**, 1 (1998).
- 3) Ohsawa, K., Imai, Y., Kanazawa, H., Sasaki, Y. and Kohsaka, S.: *J. Cell Sci.*, **113**, 3073 (2000).
- 4) Sasaki, Y., Ohsawa, K., Kanazawa, H., Kohsaka, S. and Imai, Y.: *Biochem. Biophys. Res. Commun.*, **286**, 292 (2001).
- 5) Kanazawa, H., Ohsawa, K., Sasaki, Y., Kohsaka, S. and Imai, Y.: *J. Biol. Chem.*, **277**, 20026 (2002).

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Anti Iba1 for Immunocytochemistry (Wako Cat.#019-19741)

Application for paraffin section

- 1) Deparaffinization
 - 100% xylene for 10 minutes, 2 times
 - 100% ethanol for 5 minutes, 2 times
 - 80% ethanol for 5 minutes
 - 70% ethanol for 5 minutes
 - dH₂O, 5 minutes, 2 times
- 2) Wash with 0.01M PBS, 2 times
- 3) Incubate in 0.3% H₂O₂ in methanol for 30 minutes
- 4) Wash with 0.01M PBS, 3 times
- 5) Incubate with blocking buffer (1.5% normal goat serum and 1% BSA in 0.001M PBS) for 2 hours at room temperature (RT)
- 6) Incubate with **Anti Iba1 Antibody (0.5 µg/mL)(Wako Catalog No. 019-19741)** in blocking buffer overnight at 4°C.
- 7) Wash with 0.01M PBS, 3 times
- 8) Incubate with Biotinylated anti-Rabbit IgG Antibody (1:200) in blocking buffer for 1hour at RT.
- 9) Wash with 0.01M PBS, 3 times
- 10) Incubate with Elite ABC reagent (Reagent A(1:50) and Reagent B (1:50) in 0.01 M PBS) for 1hour at RT
- 11) Wash with 0.01M PBS, 3 times
- 12) Incubate in peroxidase solution
(0.01% hydrogen peroxide and 0.05% DAB in 0.05M Tris buffer)

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