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Wako

Code No. 012-26723 (10 μL)
016-26721 (50 μL)

Anti Iba1, Monoclonal Antibody (NCNP24)
抗 Iba1，モノクローナル抗体（NCNP24）

【Background】

Iba1 is a protein highly expressed in microglia and macrophages with a molecular weight of about 16.7 kDa[1]. The protein is a commonly known microglial marker in the nervous system. This item is a mouse monoclonal antibody that reacts with Iba1[2].
For research use only. Not for use in diagnostic procedure.

【Description】

[Purification]	Purified from the hybridoma supernatant by Protein A-affinity chromatography
[Reactivity]	Reacts with Iba1
[Antigen]	Synthetic peptide corresponding to the C-terminus of Iba1
[Clone No.]	NCNP24
[Species cross reactivity]	Mouse, rat and marmoset (Other species have not been tested)
[Host]	Mouse
[Isotype]	IgG1・κ
[Concentration]	Indicated on the label.
[Formulation]	1×TBS (pH 7.4) aqueous solution with 50% glycerol containing 0.05% sodium azide.

【Applications】

Immunohistochemistry (mouse, rat and marmoset frozen section*, DAB**) 1 : 500～2,000
Immunohistochemistry (rat frozen section*, fluorescent**) 1 : 100

＊Paraffin section is not stained.
＊＊For fluorescent staining, amplify the signal using biotinylated anti-mouse IgG and streptavidin-conjugated fluorescent dye (see recommended protocol). When using a secondary antibody labeled with a fluorescent dye, the positive signal weakens.

Optimal concentration should be determined by each laboratory for each application.

【Storage】

Store at −20℃.
Avoid repeated freeze and thaw.

【Package】

012-26723	10 μL
016-26721	50 μL

【Recommended protocol (frozen section, DAB)】

1. Mouse and/or rat are perfusion-fixed with 4% paraformaldehyde. replaced sucrose, and prepared 50 μm brain section by microtome.
2. Wash : 0.3% TritonX-100 in PBS, 5 min×3
3. Block of endogenous peroxidase : 3% hydrogen peroxide in 80% methanol, 20 min, −20℃
4. Wash : 0.3% TritonX-100 in PBS, 5 min×3
5. Blocking : 1% BSA, 0.3 % TritonX-100 in PBS, 2 hours, room temperature.
6. Primary antibody : Anti-Iba1 Mouse Monoclonal Antibody (NCNP24) (1 : 500), 1% BSA, and 0.3% TritonX-100 in PBS, Overnight, 4℃
7. Wash : PBS, 5 min×3
8. Secondary antibody : biotinylated anti-mouse IgG (1 : 200), 1% BSA, and 0.3% TritonX-100 in PBS, 2 hours, room temperature
9. Wash : PBS, 5 min×3
10. ABC : VECTASTAIN ABC kit (Vector Laboratory), 2 hours, room temperature
11. Wash : PBS, 5 min×3
12. DAB solution (10 mg/15 mL TBS)
13. Wash : PBS, 5 min×3
14. Mount

【Recommended protocol (frozen section, fluorescent)】

1. Rat is perfusion-fixed with 4% paraformaldehyde. replaced sucrose, and prepared 50 μm-brain section by microtome.
2. Wash : 0.3% TritonX-100 in PBS, 5 min×3
3. Blocking : 1% BSA and 0.3% TritonX-100 in PBS, 2 hours, room temperature
4. Primary antibody : Anti Iba1, Mouse Monoclonal Antibody (NCNP24) (1 : 100), 1% BSA, and 0.3% TritonX-100 in PBS, overnight, 4℃
5. Wash : 0.3% TritonX-100 in PBS, 5 min×3
6. Secondary antibody : biotinylated anti-mouse IgG (1 : 200), 1% BSA, and 0.3% TritonX-100 in PBS, 2 hours, room temperature
7. Wash : 0.3% TritonX-100 in PBS, 5 min×3
8. Streptavidin Alexa Fluor 488 (1 : 1,000), 1% BSA, and 0.3% TritonX-100 in PBS, 2 hours, room temperature
9. Wash : 0.3% TritonX-100 in PBS, 5 min×3
10. Mount

【References】

1) Imai, Y., Ibata, I., Ito, D., Ohsawa, K. and Kohsaka, S. : *Biochem. Biophys. Res. Commun.*, **224**, 855 (1996).
2) Daniel Lee, C. Y. *et al.* : *Neuron*, **97**, 5, 1032 (2018).

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