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Wako

Code No. 019-19741

Anti Iba1, Rabbit(for Immunocytochemistry)
抗 Iba1，ウサギ（免疫細胞化学用）

Rabbit Anti Iba1 antibody is raised against synthetic peptide corresponding to C-terminus of Iba1.
For Research Use Only. Not for use in diagnostic procedures or therapeutic use.

Preparation : Purified by the antigen affinity chromatography from rabbit antisera and prepared in TBS solution. Contains no preservatives and stabilizers.
Specificity : Reactive with mouse and rat Iba1. (Other species have not been tested)
Working Concentration : Immunocytochemistry 1-2 μg/mL
Immunohistochemistry (frozen section) 0.5-1 μg/mL
Storage : Keep at −20℃.
After opening aliquot contents and freeze at −20℃.
Package : 50μg(100μL)

Recommended protocol(Immunohistochemistry, frozen section)
Rat or mouse was perfusion-fixed with 4% paraformaldehyde. replaced sucrose, and prepared 20-50 μm brain section by microtome.

Wash : 0.3% TritonX-100 in PBS, 5 min×3
↓
Blocking : 1% BSA, 0.3% TritonX-100/PBS, 2 hours, RT
↓
Primary antibody : Rabbit anti-Iba1(1：1000), 1% BSA, and 0.3% TritonX-100 in PBS, Over night, 4℃
↓
Wash : 0.3% TritonX-100 in PBS, 5 min×3
↓
Secondary antibody : AlexaFluor®488 anti-rabbit IgG(1 : 1000), 1% BSA, and 0.3% TritonX-100 in PBS, 2 hours, RT
↓
Wash : 0.3% TritonX-100 in PBS, 5 min×3
↓
Mount

Trouble Shooting
We confirm this product has as good quality as that of the previous lot by immunostaining test. Since this product is polyclonal antibody, best assay conditions vary depending on the activity of each lot. Therefore we recommend you to consider the optimal conditions each time.

Problem	Possible cause	Solution
No signal	1st and/or 2nd antibody NOT added or enough.	Check the 1st antibody and 2nd antibody, please add.
	2nd antibody is not compatible	Use a 2nd antibody against rabbit IgG.
	In case of immunofluorescence method, a suitable fluorescent filter is not used.	Select a suitable filter for the using fluorescent dye.
Weak signal	Perfusion-fixation is not done.	Perform perfusion-fixation.
	The antigens are denatured.	Do antigen retrieval-by using A or B buffer. (A) Citrate Buffer(pH 6.0), 90℃, 9 min (B) TE buffer(pH 9.0), 90℃, 9 min
	The section is old or broken.	Make new section. 50 μm thickness section works well.
	In case of Immunofluorescence method, the laser intensity of fluorescence microscope is too weak.	Adjust the laser intensity.
Stain neurons and microglia	The concentration of Anti Iba1 antibody is too high.	Titrate the antibody to the optimal concentration. Recommended dilution range is 1 : 500-1,000
	The concentration of 2nd antibody is too high.	Titrate the antibody to the optimal concentration.
	The reaction time of 2nd antibody is too long.	Recommended reaction time is 1-2 hour.
	The antigens are denatured.	Do antigen retrieval by using A or B buffer. (A) Citrate Buffer(pH 6.0), 90℃, 9 min (B) TE buffer(pH 9.0), 90℃, 9 min
High background	Blocking is absent or insufficient.	Extend the blocking incubation time. Try another blocking solution. (Wako recommends PBS with 1% BSA, 0.3% Tween-20 or 3% normal serum of 2nd antibody's host).
	The concentration of 2nd antibody is too high.	Titrate the antibody to the optimal concentration.
	The reaction time of 2nd antibody is too long.	Recommended reaction time is 1-2 hour.
	The section is old or broken.	Make new section. 50 μm thickness section works well.

Problem	Possible cause	Solution
High background	The antigens are denatured.	Do antigen retrieval by using A or B buffer. (A) Citrate Buffer (pH 6.0), 90℃, 9 min (B) TE buffer (pH 9.0), 90℃, 9 min
	In case of immunoenzyme method, reaction time of substrate is too long.	Shorten reaction time.
	In case of using biotin conjugated antibody, endogenous peroxidases are active.	Inactivate endogenous peroxidases. Before blocking, incubate with 3% H_2O_2, 80%Methanol, -20℃, 20 min.
Staining clearer in rat than in mouse.	This antibody's property	This product tends to stain clearer in rat than in mouse (for unknown reasons).

References :

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3) Ohsawa, K., Imai, Y., Kanazawa, H., Sasaki, Y. and Kohsaka, S. : *J. Cell Sci*., **113**, 3073 (2000).
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FUJIFILM Wako Pure Chemical Corporation

1-2, Doshomachi 3-Chome, Chuo-Ku, Osaka 540-8605, Japan
Telephone : +81-6-6203-3741
Facsimile : +81-6-6201-5964
http://ffwk.fujifilm.co.jp

FUJIFILM Wako Chemicals U.S.A. Corporation

1600 Bellwood Road
Richmond, VA 23237
U.S.A.
Telephone : +1-804-271-7677
Facsimile : +1-804-271-7791
http://www.wakousa.com

FUJIFILM Wako Chemicals Europe GmbH

Fuggerstrasse 12
D-41468 Neuss
Germany
Telephone : +49-2131-311-0
Facsimile : +49-2131-311100
http://www.wako-chemicals.de

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