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.

This product is for laboratory use only; use in any such application is the responsibility of the user.

Preparation : Purified by the antigen affinity chromatography from rabbit antisera and prepared in TBS solution. Contains no preservatives and stabilizers.

Specificity : Specific to microglia and macrophage, but not cross-reactive with neuron and astrocyte.
Reactive with human, mouse and rat Iba1.

Working Concentration : Immunocytochemistry 1-2 μg/mL
Immunohistochemistry 0.5-1 μg/mL

Storage : Keep at −20℃.
After opening aliquot contents and freeze at −20℃.

Package : 50 μg(100 μL)

Recommended protocol(IHC, frozen section)

Rat or mouse was perfusion-fixed with 4% paraformaldehyde. replaced sucrose, and prepared 50 μm brain section by microtome.

Wash : 0.3 % TritonX-100 in PBS, 5 min×3
↓
Blocking : 1 % BSA, 0.3 % TritonX-100/PBS, 2hour, 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, 2hour, 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(pH6.0), 90℃, 9min (B) TE buffer(pH9.0), 90℃, 9min
	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(pH6.0), 90℃, 9min (B) TE buffer(pH9.0), 90℃, 9min
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(pH6.0), 90℃, 9min (B) TE buffer(pH9.0), 90℃, 9min
	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℃, 20min.
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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