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Value from Innovation

Wako

FUJIFILM Wako Pure Chemical Corporation

Microglia Research Products (Ver.2)

- Fundamentals of Microglia
- Immunohistochemistry of Microglia
- Antibodies
- ELISA Kits
- Bioactive Agents

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What are Microglia?

Microglia are glial cells responsible for immune function in the central nervous system. They were first described and named by the Spanish neuroscientist Pío del Río Hortega in 1919[1].

Unlike neurons, astrocytes, and oligodendrocytes, which are derived from the ectoderm, microglia are derived from mesodermal progenitors in the yolk sac, erythro-myeloid progenitors (EMPs)[2]. EMPs generated early in development migrate throughout the body, and those that migrate to the central nervous system differentiate into microglia. Later, as development progresses, EMPs are replaced in most tissues by monocyte-derived macrophages of hematopoietic stem cell (HSC) origin. However, these macrophages cannot readily penetrate the blood-brain barrier formed during development, and replacement with macrophages of HSC origin rarely occur in the central nervous system[3].

Like macrophages, microglia phagocytose debris and dead cells and release chemokines and cytokines. These activities quickly remove unwanted materials such as debris and dead cells, and repair of the injured site begins following an immune response. Microglia are thus responsible for maintaining homeostasis in the central nervous system. However, with advanced neurodegenerative disease, severe neuronal injury, or chronic inflammatory responses, microglia can exacerbate the disease or injury. Microglia-targeted drug discovery not only makes use of the intrinsic function of microglia, but also attempts to block the involvement of microglia in the exacerbation of symptoms.

The morphological changes in microglia depend significantly on the environment. Normally, microglia are in a resting (ramified) form with processes extending from a relatively small cell body. However, when activated by nerve injury or other stimuli, their morphology changes to an amoeboid form with an enlarged cell body and retracted processes, resembling macrophages.

Previous studies have shown two types of activated microglia: M1 (pro-inflammatory) and M2 (anti-inflammatory) microglia[4]. The former responds to IFN-γ, TNF-α, and damage-associated molecular patterns (DAMPs) and releases pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and reactive oxygen species, while the latter responds to IL-4 and TGF-β and releases anti-inflammatory cytokines such as IL-10 and TGF-β, as well as trophic factors such as BDNF[5] (Figure 1). In recent years, researchers have attempted to extend the classification of microglia into subtypes. In a 2019 study, mouse microglia were analyzed by single cell RNA-seq and divided into at least nine different clusters[6].

Microglia also play other important roles in the development and function of the central nervous system, such as extending their processes to the synapses of neurons to monitor neural status through direct contact[7] and being involved in synaptic pruning[8].

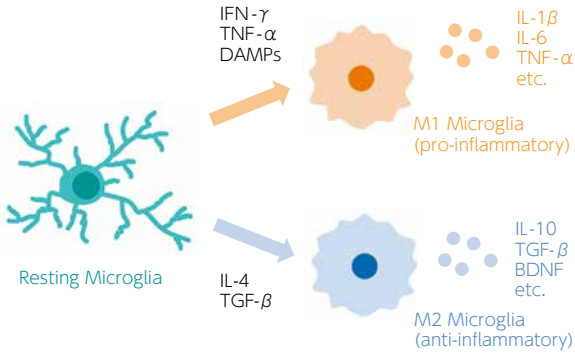

Figure 1. Microglial activation and related factors

Markers of Microglia

In microglial research, it is necessary to distinguish microglia from neurons and other glial cells. Previous studies have identified microglia-specific markers, many of which are shared with macrophages (Figure 2). It should be noted that each marker is expressed differently in different microglial subtypes, and their expression is altered by activation and other factors.

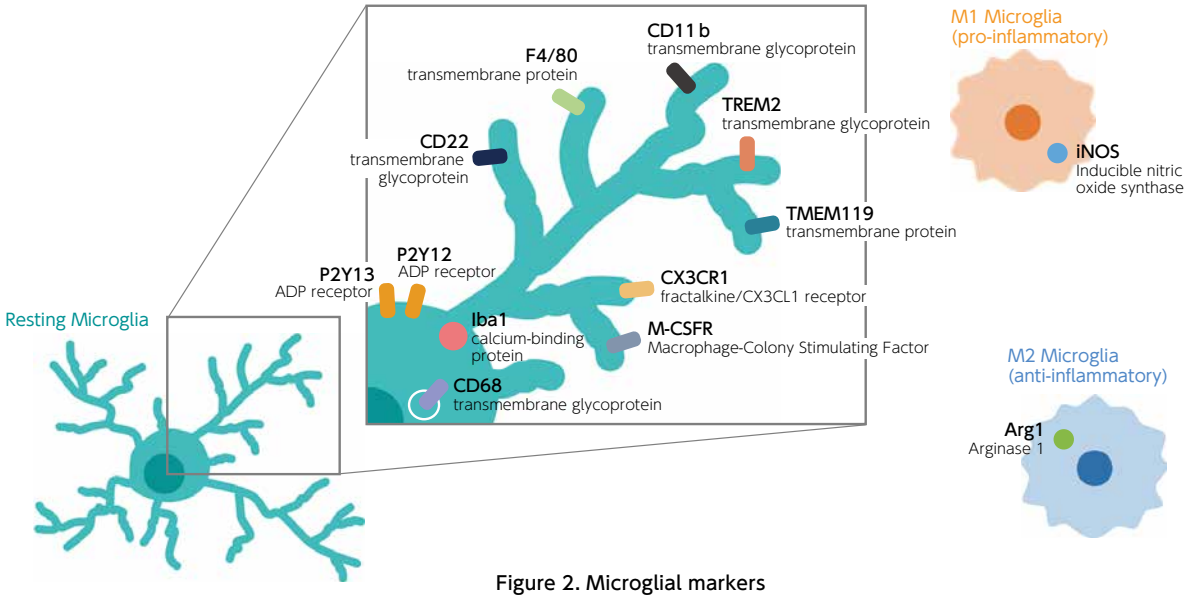

Figure 2. Microglial markers

Microglia and Neurological and Psychiatric Diseases

Microglia are associated with various neurological and psychiatric disorders and have attracted attention as a target for drug discovery. Microglia that respond specifically to disease are called disease-associated microglia (DAM).

Alzheimer's disease

The amyloid hypothesis has been proposed to explain the pathogenesis of Alzheimer's disease. It is known that microglia are activated around β-amyloid (Aβ) aggregates in the brains of Alzheimer's disease patients[9]. While microglia phagocytose Aβ and contribute to the suppression of Alzheimer's disease, cytokines and reactive oxygen species released by microglia contribute to the progression of Alzheimer's disease. Thus, microglia both suppress and promote the progression of Alzheimer's disease (Figure 3).

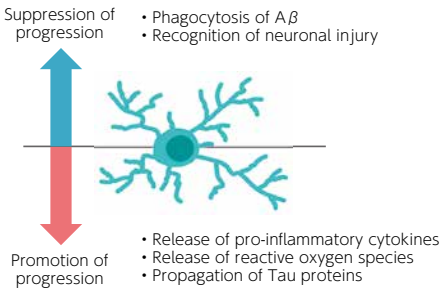

Figure 3.
Alzheimer's disease and Microglia

In 2013, the R47H mutation in TREM2, expressed in microglia, was reported as a risk factor for Alzheimer's disease[10),11)], and the link between microglia and Alzheimer's disease became a focus of attention. In a mouse model with mutations associated with familial Alzheimer's disease (the 5xFAD mouse model), knockout of TREM2 increases Aβ . Based on its ability to sense lipids associated with Aβ accumulation and cell injury, TREM2 is believed to play an important role in detecting and responding to abnormalities in the central nervous system[12)].

Neurofibrillary tangles, one of the hallmarks of Alzheimer's disease, are caused by the tau protein. Exosomes released from microglia are involved in the interneuronal propagation of tau protein[13)].

Some researchers consider microglia as a therapeutic target for Alzheimer's disease. Various approaches are being developed, such as promoting phagocytosis of Aβ by microglia and inhibiting neuroinflammation induced by microglia.

Parkinson's disease

Microglia are activated in Parkinson's disease patients, and elevated pro-inflammatory cytokines such as IL-1β and TNF-α are found in the striatum of patients[14)]. In a mouse model of Parkinson's disease, microglial infiltration is observed in the substantia nigra, and inhibition of microglial activation can suppress cell death in dopaminergic neurons[15)]. These reports suggest that neuroinflammation mediated by microglia is strongly associated with the pathogenesis and progression of Parkinson's disease.

Depression

Several hypotheses for the pathogenesis of depression are under consideration, including the monoamine hypothesis and the BDNF hypothesis. Inflammatory responses in the brain are also thought to be involved in the pathogenesis of depression (the inflammatory hypothesis of depression). It has long been known that depressive symptoms frequently occur in patients who have undergone immunotherapy with cytokines[16)], and inflammatory markers are expressed at high levels in patients with depression[17)].

Microglia produce or respond to pro-inflammatory cytokines in the brain and play a central role in the inflammatory hypothesis of depression. Selective serotonin reuptake inhibitors (antidepressants) inhibit microglial activation[18)], and BDNF, the expression of which is increased by antidepressants, is also released from microglia. Accordingly, the role of microglia needs to be clarified to understand the pathogenesis of depression.

Autism

Autism is thought to be caused in part by abnormal synapse formation between neurons. Microglia are involved in the formation of neural circuits and the removal of synapses, and it has been suggested that abnormal synapse formation due to microglial dysfunction may be a cause of autism.

In mice, knockout of *Fmr1*, a risk gene for autism, resulted in reduced phagocytosis of synapses by microglia[19)]. Knockout of *Cx3cr1*, which is expressed in microglia, led to inadequate synaptic pruning by microglia, resulting in reduced connectivity in brain regions associated with autism and stereotypical autistic behaviors (repetitive behaviors)[20)].

References

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Immunohistochemistry of Microglia

Introduction

Fujifilm Wako's “Anti Iba1, Rabbit (for Immunocytochemistry)” is an excellent microglial marker antibody since it can stain even microglial processes. This page describes the protocol and points to note when performing microglial immunohistochemistry. Frozen sections of mouse brain and a fluorescent dye are used as an example.

Standard Protocol

1. Preparation of tissue sections

- ☐ Mice are perfused and fixed in 4% paraformaldehyde-phosphate buffer (①).
- ☐ Replace with sucrose, and prepare frozen blocks.
- ☐ prepare 50 µm-sections in thickness (②) using a microtome.

2. Washing – Blocking

- ☐ Wash with 0.3% TritonX-100[b]/PBS[c] 3 times for 5 minutes each.
- ☐ Block in 1% BSA[d], 0.3% TritonX-100/PBS for 2 hours at room temperature (③).

3. Primary antibody reaction

- ☐ Add Anti Iba1, Rabbit (for immunohistochemistry)[e] to 1% BSA, 0.3% TritonX-100/PBS at 1:1,000 dilution (④).
- ☐ Incubate overnight at 4℃ (⑤).

4. Washing

- ☐ Wash with 0.3% TritonX-100/PBS 3 times for 5 minutes each.

5. Secondary antibody reaction

- ☐ Add fluorescent-labeled anti-rabbit IgG antibody[f] to 1% BSA, 0.3% TritonX-100/PBS at 1:1,000 dilution (⑥).
- ☐ Incubate for 2 hours at room temperature (⑦).

6. Washing

- ☐ Wash with 0.3% TritonX-100/PBS 3 times for 5 minutes each (⑧).

7. Mounting

- ☐ Mount the sections in mounting media.

8. Observation

- ☐ Observe the sections under a fluorescence microscope or a confocal microscope.

Reagents

a. 4% Paraformaldehyde Phosphate Buffer Solution
(Product Number: 163-20145)
b. Polyoxyethylene(10) Octylphenyl Ether
(Product Number: 169-21105)
c. 1 × PBS (－)
(Product Number: 164-25511)
PBS(-), Powder, for 1 L
(Product Number: 164-28713)
10 × PBS (－)
(Product Number: 163-25265/314-90185)
d. Albumin, from Bovine Serum (BSA), Globulin Free
(Product Number: 019-15101)
e. Anti Iba1, Rabbit (for Immunocytochemistry)
(Product Number: 019-19741)
f. Alexa Fluor® 488-AffiniPure Goat Anti-Rabbit IgG (H+L)
(Jackson ImmunoResearch, 111-545-144)
Alexa Fluor® 647-AffiniPure Donkey Anti-Rabbit IgG (H+L)
(Jackson ImmunoResearch, 711-605-152)
g. Polyoxyethylene(20) Sorbitan Monolaurate
(Product Number: 162-21112)
h. Prepared by mixing the following reagents
Citric Acid Monohydrate
(Product Number: 031-03492)
Trisodium Citrate Dihydrate
(Product Number: 197-01782)
i. Prepared by mixing the following reagents (pH adjustment required)
1 M Tris-HCl (pH 8.0)
(Product Number: 314-90065)
0.5 M EDTA (pH 8.0)
(Product Number: 311-90075)

Point

① Samples without perfusion fixation or with inadequate fixation result in poor staining. Perfusion fixation with 4% paraformaldehyde-phosphate buffer is recommended.
② Recommended thickness of tissue sections is 20-50 µm.
③ If background is high, try extending the incubation time for blocking or changing the blocking solutions. The following blocking solutions can also be used:
- 1% BSA, 0.3% Tween-20/PBS
- 3% normal serum of the host of the secondary antibody

④ If the staining is weak, increase the antibody concentration. If the background is high, decrease the antibody concentration. Recommended dilution is 1:500-1,000.
⑤ Although it depends on the sample, rat cerebellum has been successfully stained with just 2 hours of incubation.
⑥ If the staining is weak, increase the antibody concentration. If the background is high, decrease the antibody concentration. Recommended dilution is 1:500-1,000.
⑦ If the background is high, shorten the incubation time for the secondary antibody. Recommended incubation time is 1-2 hours.
⑧ If the background is high, increase the number of washes.

If microglia do not stain well using the techniques described at left, perform antigen retrieval after sectioning using one of the following:
(A) Citrate buffer (pH 6.0)[h] for 9 minutes at 90℃
(B) TE buffer[i] (pH 9.0) for 9 minutes at 90℃

Iba1

Iba1 (Ionized calcium-binding adapter molecule 1) is an approximately 17 kDa calcium-binding protein. It is used as a microglial marker because it is expressed specifically in microglia in the central nervous system[1]. It is expressed in both resting and activated microglia, but is reportedly expressed more highly in activated microglia[2]. It is also expressed in macrophages in peripheral tissues and is known as AIF-1 (Allograft inflammatory factor-1).
Iba1 binds to F-actin in cells to form actin bundles. The formation of actin bundles is thought to be required for the membrane ruffling observed during cell migration and phagocytosis[3].

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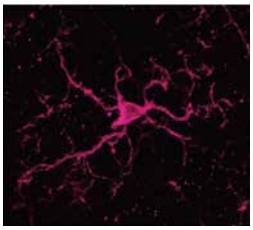
Anti Iba1 Antibodies of Fujifilm Wako

Anti Iba1, Rabbit (for immunocytochemistry)

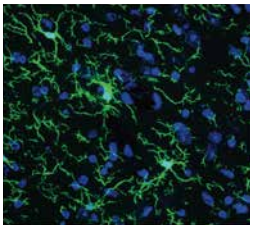
"Anti Iba1, Rabbit (for immunocytochemistry)" (Product No. 019-19741), which allows even microglia processes to be stained by immunohistochemical staining, is used by researchers all over the world as a standard for microglia marker antibody. It has been appearing in an increasing number of publications, including many articles published in top journals (Nature, Cell, Neuron, etc.), and the annual number of such publications exceeded 1,300 in 2022.



Immunohistochemical staining of mouse cerebellum

Development of rabbit monoclonal antibody

In 2023, Fujifilm Wako launched a rabbit monoclonal antibody, "Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant" (Product No. 018-28523). We have confirmed that this antibody has the same performance as "Anti Iba1, Rabbit (for immunocytochemistry)" in Immunohistochemical staining of mice and rat brains. It has also been reported to obtain good results in immunohistochemical staining of mouse retinas.



Immunohistochemical staining of mouse cerebral cortex

Product Lineup

Host	Rabbit								Goat	Mouse	
Product Number	018-28523	019-19741	013-27691	016-20001	016-26461	015-28011	012-28401	013-26471	011-27991	016-26721	013-27593
Discription	Rabbit monoclonal antibody	For Immuno-staining (Standard)	For paraffin section	For western blotting	Biotin-conjugated	SPICA Dye™ 568-conjugated	SPICA Dye™ 594-conjugated	Red fluorochrome (635)-conjugated	Goat polyclonal antibody	Mouse monoclonal antibody (NCNP24)	Mouse monoclonal antibody (NCNP27)
Antibody	Monoclonal antibody	Polyclonal antibody								Monoclonal antibody	
Conjugate	Unconjugated	Unconjugated	Unconjugated	Unconjugated	Biotin	SPICA Dye™ 568 Ex=556 nm Em=591 nm	SPICA Dye™ 594 Ex=575 nm Em=611 nm	Red fluorochrome (635) Ex=634 nm Em=654 nm	Unconjugated	Unconjugated	Unconjugated
Concentration (mg/mL)	1.0-1.2	0.5-0.7	0.5-0.7	0.5-0.7	0.5-0.6	0.5-0.6	0.5-0.6	0.5-0.6	0.6-0.7	0.9-1.6	0.9-1.3
Antigen	Synthetic peptide (Iba1 C-terminal sequence)										
Cross-reactivity	Mouse Rat	Human Mouse Rat Other*1	Mouse Rat	Human Mouse Rat	Mouse Rat	Mouse Rat	Mouse Rat	Mouse Rat	Mouse Rat	Marmoset Mouse Rat	Human
Application Figures indicate recommended concentrations	IHC (F) 1:200-10,000 FCM 1:100-10,000	IHC (F) 1:500-1,000 ICC 1:500-1,000	IHC (P) 1:500-1,000	WB 1:500-1,000	IHC (F) 1:200-2,000	IHC (F) 1:200-2,000	IHC (F) 1:200-2,000	IHC (F) 1:200-2,000	IHC (F) 1:250-1,000 IHC (P) 1:250-1,000 WB 1:1,000	IHC (F, DAB) 1:500-2,000 IHC (F, fluorescent) 1:100	IHC (P, DAB) 1:100-1,000
Package Size	100 µL	50 µg	50 µg	50 µg	100 µL	100 µL	100 µL	100 µL	100 µL	50 µL	50 µL

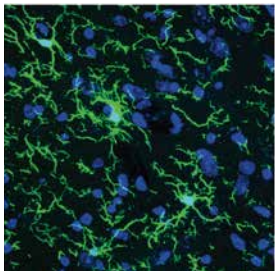
* 1 Experience with dog, cat, pig, marmoset, and zebrafish, etc. has been reported.

[Abbreviations] FCM: Flow cytometry, IHC(F): Immunohistochemistry (frozen section), IHC(P): Immunohistochemistry (paraffin section), ICC: Immunocytochemistry, WB: Western blotting

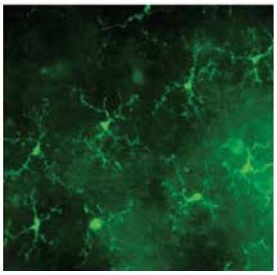
Application Data

Immunohistochemistry

Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant

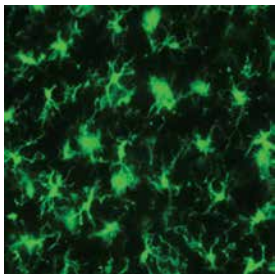


Species: Mouse
Site: Cerebral cortex
Sample: Frozen section
Antibody concentration: 1:1,000



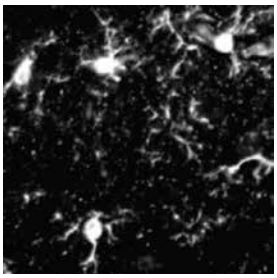
Species: Mouse
Site: Retina (Flat-mount)
Sample: Frozen section
Antibody concentration: 1:2,000
[Data by courtesy of]
Dr. Watanabe, Dr. Iwagawa,
University of Tokyo Hospital

Anti Iba1, Rabbit (for immunocytochemistry)



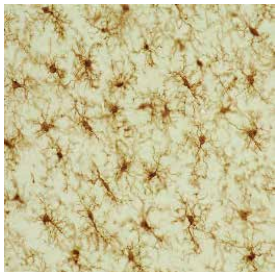
Species: Rat
Site: Cerebral cortex
Sample: Frozen section
Antibody concentration: 1:1,000
[Data by courtesy of]
Dr. Sanagi, Dr. Manabe, Dr. Ichinohe,
Dr. Kohsaka
National Center of Neurology and Psychiatry

Anti Iba1, Rabbit (for paraffin section)



Species: Rat
Site: Hippocampus vicinity
Sample: Paraffin section
Antibody concentration: 1:1,000

Anti Iba1, Rabbit, Biotin-conjugated



Species: Rat
Site: Cerebral cortex
Sample: Frozen section
Antibody concentration: 1:200
[Data by courtesy of]
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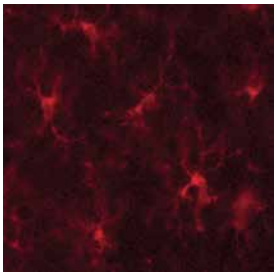
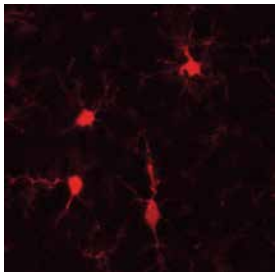
Anti Iba1, Rabbit, SPICA Dye™ 568-conjugated



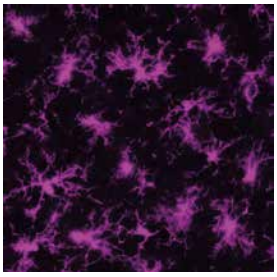
Species: Rat
Site: Cerebral cortex
Sample: Frozen section
Antibody concentration: 1:200

Anti Iba1, Rabbit, SPICA Dye™ 594-conjugated



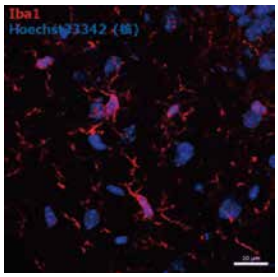
Species: Rat
Site: Cerebral cortex
Sample: Frozen section
Antibody concentration: 1:200

Anti Iba1, Rabbit, Red Fluorochrome (635)-conjugated



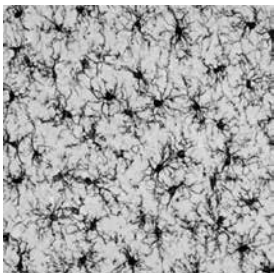
Species: Rat
Site: Cerebral cortex
Sample: Frozen section
Antibody concentration: 1:200
[Data by courtesy of]
Dr. Sanagi, Dr. Manabe, Dr. Ichinohe,
Dr. Kohsaka
National Center of Neurology and Psychiatry

Anti Iba1, Goat



Species: Mouse
Site: Hippocampus
Antibody concentration: 1:200
[Data by courtesy of]
Dr. Takata
Division of Integrated Pharmaceutical
Sciences, Kyoto Pharmaceutical University

Anti Iba1, Mouse Monoclonal Antibody (NCNP24)



Species: Rat
Site: Cerebral cortex
Sample: Frozen section
Antibody concentration: 1:1,000
[Data by courtesy of]
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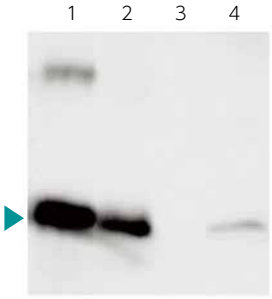
Dr. Kohsaka
National Center of Neurology and Psychiatry

Western blotting

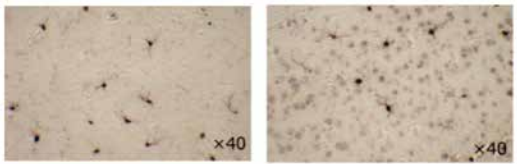


Anti Iba1, Rabbit (for Western blotting)

1. Iba1 protein 10 ng
2. Rat Microglia 10 μg
3. Rat Neuron 10 μg
4. Rat cerebral cortex 150 μg

SDS-PAGE: 5.5% stacking gel, 12.5% running gel, 100V
Blocking: 3% skim milk/TBS, 1 hour, room temperature.
Primary antibody: 1:1,000 concentration, 3% skim milk/TTBS, overnight, 4℃
Secondary antibody: Peroxidase-labeled Anti Rabbit IgG (1:5,000), 3% skim milk/TTBS, 1 hour, room temperature

Comparative Data of Anti Iba1, Goat (DAB staining)

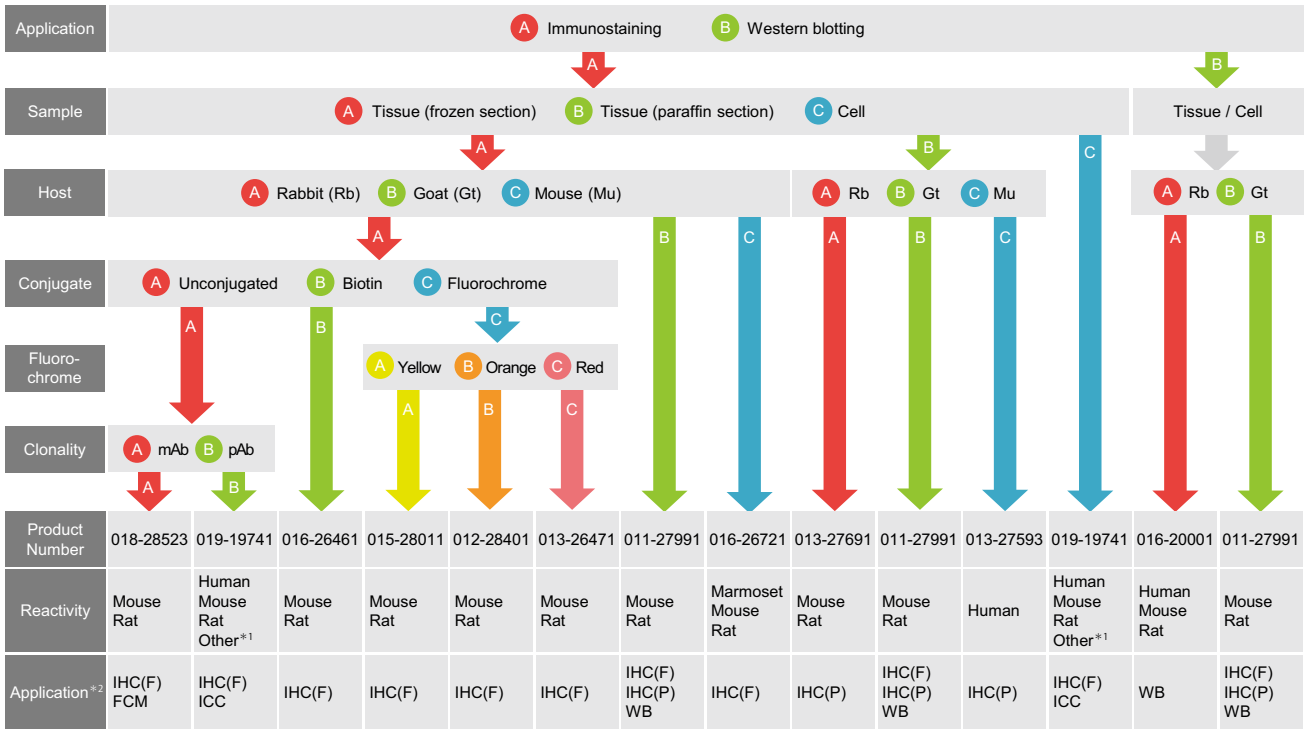


Fujifilm Wako　　Competitor A

Sample: paraffin section of mouse brain frontal lobe
1st antibody: Anti Iba1, Goat (1:1,000)
2nd antibody: Anti goat IgG, biotin-conjugated
Antigen retrieval: 10 mM citric acid buffer(pH 6.0), 90℃, 10 min

Result　Fujifilm Wako's Anti Iba1, Goat showed less non-specific signals than Competitor A.

Selection Flowchart



* 1 Experience with dog, cat, pig, marmoset, and zebrafish, etc. has been reported.
* 2 [Abbreviations] FCM: Flow cytometry, IHC(F): Immunohistochemistry (frozen section), IHC(P): Immunohistochemistry (paraffin section), ICC: Immunocytochemistry, WB: Western blotting

Product Number	Product Name	Grade	Package Size
018-28523	Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant	for Immunochemistry	100 μL
019-19741	Anti Iba1, Rabbit (for Immunocytochemistry)		50 μg
013-27691	Anti Iba1, Rabbit (for Paraffin Section)		50 μg
016-20001	Anti Iba1, Rabbit (for Western Blotting)		50 μg
016-26461	Anti Iba1, Rabbit, Biotin-conjugated		100 μL
015-28011	Anti Iba1, Rabbit, SPICA Dye™ 568-conjugated		100 μL
012-28401	Anti Iba1, Rabbit, SPICA Dye™ 594-conjugated		100 μL
013-26471	Anti Iba1, Rabbit, Red Fluorochrome(635)-conjugated		100 μL
011-27991	Anti Iba1, Goat		100 μL
016-26721	Anti Iba1, Monoclonal Antibody (NCNP24)		50 μL
013-27593	Anti Human Iba1, Monoclonal Antibody (NCNP27)		50 μL

Cytokines

Activated microglia release a variety of cytokines. Cytokines play an important role in regulating immune responses. Excessive cytokines, however, are known to cause neuronal damage, leading to the development or exacerbation of various neurological and psychiatric disorders. Fujifilm Wako offers a lineup of ELISA kits for cytokines.

Human

Analyte	IFN-γ	IL-6	IL-8 (CXCL8)	MCP-1 (CCL2)	TNF-α
Product Number	631-47891	635-42311	632-42321	638-53411	639-42331
Analysis sample	Serum Plasma (EDTA/Heparin) Culture supernatant	Serum Plasma (EDTA recommended) Culture supernatant	Serum Plasma (EDTA/Heparin) Culture supernatant	Serum Plasma (EDTA/Heparin) Urine Culture supernatant	Serum Plasma (EDTA/Heparin) Culture supernatant
Calibration curve range	0.768-75.0 pg/mL	1.16-500 pg/mL	0.686-500 pg/mL	3.85-500 pg/mL	2.05-500 pg/mL
Sample volume (diluted)	100 μL/well	100 μL/well	100 μL/well	100 μL/well	100 μL/well

Mouse

Analyte	IFN-γ	IL-12	MCP-1 (CCL2)	TNF-α
Product Number	630-44701	638-40841	637-54101	634-44721
Analysis sample	Serum Plasma (EDTA/Heparin)	Serum Plasma (EDTA) Culture supernatant	Serum Plasma (EDTA/Heparin) Urine	Serum Plasma (EDTA recommended)
Calibration curve range	2.05-500 pg/mL	2.87-700 pg/mL	3.85-500 pg/mL	3.58-700 pg/mL
Sample volume (diluted)	50 μL/well	100 μL/well	50 μL/well	50 μL/well

Product Number	Product Name	Package Size
631-47891	LBIS Human IFN-γ ELISA Kit	96 tests
635-42311	LBIS Human IL-6 ELISA Kit	96 tests
632-42321	LBIS Human IL-8 (CXCL8) ELISA Kit	96 tests
638-53411	LBIS Human MCP-1 (CCL2) ELISA Kit	96 tests
639-42331	LBIS Human TNF-α ELISA Kit	96 tests
630-44701	LBIS Mouse IFN-γ ELISA Kit	96 tests
638-40841	LBIS Mouse IL-12 ELISA Kit	96 tests
637-54101	LBIS Mouse MCP-1 (CCL2) ELISA Kit	96 tests
634-44721	LBIS Mouse TNF-α ELISA Kit	96 tests

Wako • blog "Front Line of Microglia Research"

Neuropathic pain
Makoto Tsuda Department of Life Innovation, Graduate School of Pharmaceutical Sciences, Kyushu University

Origin of microglia and brain diseases
Kazuyuki Takata Division of Integrated Pharmaceutical Sciences, Kyoto Pharmaceutical University

Phagocytic cells in the brain
Rena Kono, Yuji Ikegaya, and Ryuta Koyama Graduate School of Pharmaceutical Sciences, The University of Tokyo

Macrophages control the inflammation and subsequent neural repair after ischemic stroke
Kento Otani[1, 2], and Takashi Shichita[1, 3]
1 Stroke Renaissance Project, Tokyo Metropolitan Institute of Medical Science, and Core Research for Evolutional Science and Technology (CREST), Japan Agency for Medical Research and Development (AMED)
2 Division of Biochemistry, Faculty of Pharmacy and Graduate School of Pharmaceutical Science, Keio University
3 Precursory Research for Innovative Medical Care (PRIME), Japan Agency for Medical Research and Development (AMED)

Reverse translational research in psychiatry using human blood to predict brain microglial activity
Takahiro A. Kato Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University

Control of stroke via microglia-astrocyte crosstalk
Schuichi Koizumi Department of Neuropharmacology, Interdisciplinary Graduate School of Medicine, University of Yamanashi

US: https://labchem-wako.fujifilm.com/us/wako-blog/index.html
Europe: https://labchem-wako.fujifilm.com/europe/wako-blog/index.html
Asia: https://labchem-wako.fujifilm.com/asia/wako-blog/index.html

US Europe Asia

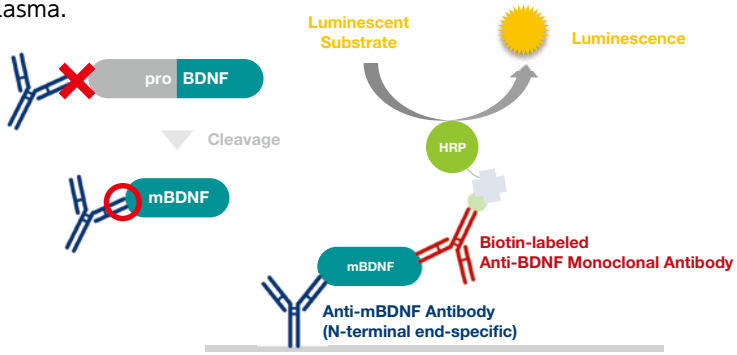
Brain-Derived Neurotrophic Factor (BDNF)

Mature BDNF ELISA Kit *Wako*, High Sensitive

Mature BDNF ELISA Kit *Wako*, High Sensitive is a sandwich ELISA that combines anti-mBDNF N-terminal end-specific monoclonal antibody and anti-BDNF monoclonal antibody. The cross-reactivity with proBDNF is substantially reduced by using end-specific antibodies, and the sensitivity is increased through use of a luminescent substrate. This kit can be used for detection mBDNF in human saliva and mouse serum or plasma.

Features

- Specifically detects mBDNF
  - Reactivity with human proBDNF: 1.30%
- Highly sensitive
  - Lower limit of calibration curve: 0.116 pg/mL



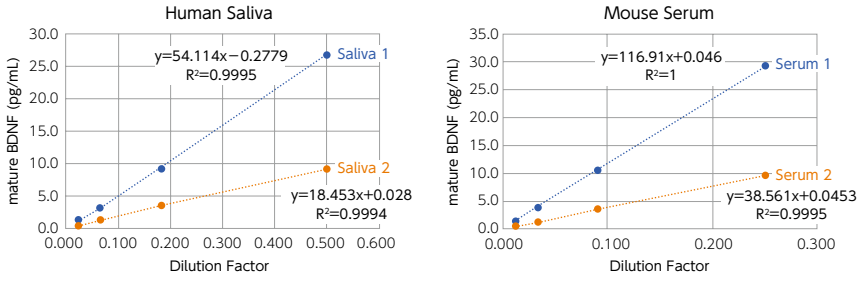
	This kit (Luminescence)	Fujifilm Wako (Chromogenic)	Competitor A	Competitor B	Competitor C
Sensitivity (lower limit of calibration curve)	0.116 pg/mL	4.1 pg/mL	62.5 pg/mL	15.6 pg/mL	15.0 pg/mL
Cross-reactivity with human proBDNF	1.30%	Approx. 10%	Approx. 10%	Approx. 15%	Approx. 50%

Product Lineup

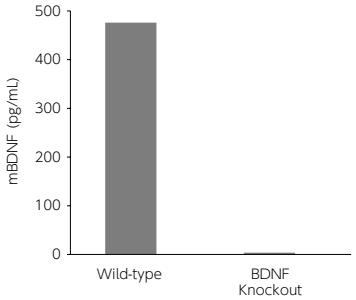
Product Name	Mature BDNF ELISA Kit *Wako*, High Sensitive	Mature BDNF ELISA Kit *Wako*
Product Number	290-85801	296-83201
Calibration curve range	0.116 - 50 pg/mL	4.1 - 1,000 pg/mL
Analyte	mBDNF	mBDNF
Reactivity with human proBDNF	1.30%	Approximately 10%
Analysis sample	Mouse Serum/Plasma/Brain lysate Rat Serum/Plasma Human Serum/Plasma/Saliva	Human Serum/Plasma
Sample volume	13 μL (4-fold dilution)	Serum : 10 μL (10-fold dilution) Plasma : 5 μL (20-fold dilution)
Measurement duration	Approx. 4 hours	Approx. 4 hours
Detection method	Luminescence (a plate reader for luminescence measurement is required)	Chromogenic

Application Data

Dilution linearity test



BDNF Measurement in brain lysate in wild-type and BDNF knockout mice



Product Number	Product Name	Grade	Package Size
290-85801	Mature BDNF ELISA Kit *Wako*, High Sensitive	for Immunochemistry	96 tests

∗ Mature BDNF ELISA Kit *Wako*, High Sensitive (Product No. 290-85801), is an upgraded kit and replaces Mature BDNF Kit *Wako*, High Sensitive (Product No. 298-83901).

Related Products

Product Number	Product Name	Grade	Package Size
296-83201	Mature BDNF ELISA Kit *Wako*	for Immunochemistry	96 tests

β Amyloid (Aβ)

β Amyloid ELISA Kit *Wako* series uses a highly specific monoclonal antibody developed by Takeda Pharmaceutical Company Limited. The kits can be used to measure not only Aβ in serum, tissue extracts, culture supernatants, and cerebrospinal fluid, but also Aβ(40) and Aβ(42) in plasma, which was difficult to measure in the past. These kits have been used in many studies.

Product Number	Product Name	Clone No.		Human				Mouse/Rat				Calibration curve range (pmol/L)
		Capture	Detection	Aβ (1-40)	Aβ (1-42)	Aβ (40)	Aβ (42)	Aβ (1-40)	Aβ (1-42)	Aβ (40)	Aβ (42)	
292-62301	Human β Amyloid(1-40) ELISA Kit *Wako*	BAN50	BA27(Fab')	○	×	×	×	×	×	×	×	1.0-100
298-64601	Human β Amyloid(1-40) ELISA Kit *Wako* Ⅱ	BAN50	BA27(F(ab')$_2$)	○	×	×	×	×	×	×	×	1.0-100
294-62501	Human/Rat β Amyloid(40) ELISA Kit *Wako*	BNT77	BA27(Fab')	○	×	○	×	○	×	○	×	1.0-100
294-64701	Human/Rat β Amyloid(40) ELISA Kit *Wako* Ⅱ	BNT77	BA27(F(ab')$_2$)	○	×	○	×	○	×	○	×	1.0-100
298-62401	Human β Amyloid(1-42) ELISA Kit *Wako*	BAN50	BC05(Fab')	×	○	×	×	×	×	×	×	1.0-100
296-64401	Human β Amyloid(1-42) ELISA Kit *Wako*, High Sensitive	BAN50	BC05(Fab')	×	○	×	×	×	×	×	×	0.1-20
290-62601	Human/Rat β Amyloid(42) ELISA Kit *Wako*	BNT77	BC05(Fab')	×	○	×	○	×	○	×	○	1.0-100
292-64501	Human/Rat β Amyloid(42) ELISA Kit *Wako*, High Sensitive	BNT77	BC05(Fab')	×	○	×	○	×	○	×	○	0.1-20

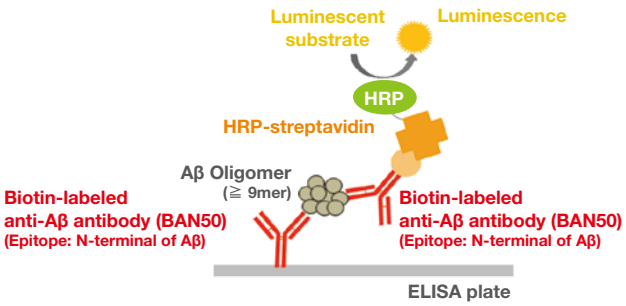
β Amyloid ELISA Kit *Wako*Ⅱ uses F(ab')$_2$ fragment antibodies for enhanced stability of the antigen-antibody reaction.
β Amyloid ELISA Kit *Wako*, high-sensitivity products are 10 times more sensitive than conventional products.
They use Fab' fragment antibodies and show low nonspecific binding.

Product Number	Product Name	Grade	Package Size
292-62301	Human β Amyloid(1-40) ELISA Kit *Wako*	for Immunochemistry	96 tests
298-64601	Human β Amyloid(1-40) ELISA Kit *Wako* Ⅱ		96 tests
294-62501	Human/Rat β Amyloid(40) ELISA Kit *Wako*		96 tests
294-64701	Human/Rat β Amyloid(40) ELISA Kit *Wako* Ⅱ		96 tests
298-62401	Human β Amyloid(1-42) ELISA Kit *Wako*		96 tests
296-64401	Human β Amyloid(1-42) ELISA Kit *Wako*, High Sensitive		96 tests
290-62601	Human/Rat β Amyloid(42) ELISA Kit *Wako*		96 tests
292-64501	Human/Rat β Amyloid(42) ELISA Kit *Wako*, High Sensitive		96 tests

High Molecular β Amyloid (Aβ Oligomer)

High Molecular Amyloid β Oligomer ELISA Kit *Wako* Ver.2 can be used to specifically measure high molecular weight Aβ oligomers of 9 or more monomers. The sandwich ELISA system uses anti-Aβ antibody (BAN50) as both the capture and detection antibody. It shows little cross-reactivity with monomer through octamer of Aβ and specifically reacts with Aβ oligomers of 9 or more monomers.

Calibration curve range (Calculated based on 16-mer MAP peptide)	0.41-100 pM (Human Cerebrospinal fluid) 0.16-40 pM (Human Serum/Plasma)
Analysis sample	Human Cerebrospinal fluid (CSF) Human Serum/Plasma (EDTA) *in vitro* Aβ Oligomer
Sample volume	Human CSF : 25 μL (4-fold dilution) 50 μL (2-fold dilution) Human Serum/Plasma : 50 μL
Measurement duration	4.5 hours
Detection Method	Luminescence (a plate reader for luminescence measurement is required)

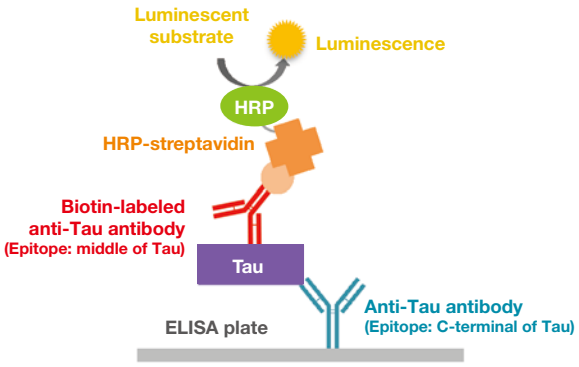


Product Number	Product Name	Grade	Package Size
290-82001	High Molecular Amyloid β Oligomer ELISA Kit *Wako* Ver.2	for Immunochemistry	96 tests

Total Tau

Tau ELISA Kit *Wako* can be used for simple measurement of total tau. Small amounts of samples (10 µL or more of human cerebrospinal fluid) can be used and provide high sensitivity.

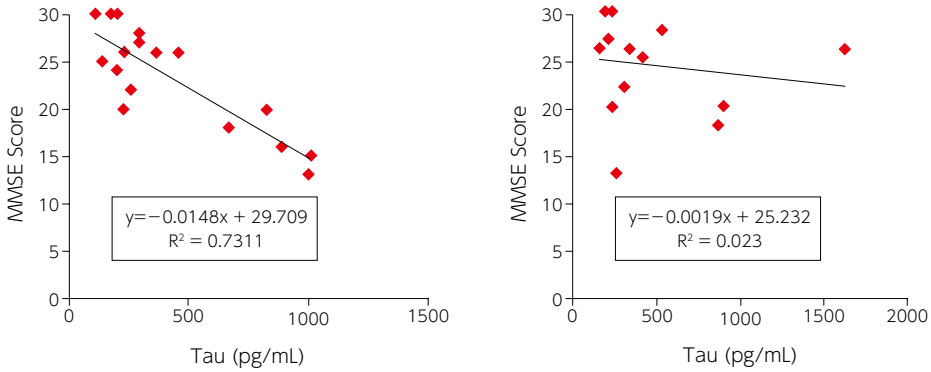
Analyte	Total Tau
Calibration curve range	4.10-1,000 pg/mL
Analysis sample	Human Cerebrospinal fluid
Sample volume	10 µL (50 µL recommended)
Measurement duration	3 hours
Detection method	Luminescence (a plate reader for luminescence measurement is required)



Correlation data with cognitive function diagnostic test (MMSE) scores

The correlation was determined between the MMSE scores* of patients and tau concentration in their cerebrospinal fluid measured by this product.

*MMSE scores are grouped as follows: 23 point or less: suspected dementia, 24-27 points: suspected mild cognitive impairment, 28-30 points: normal cognition.



Result

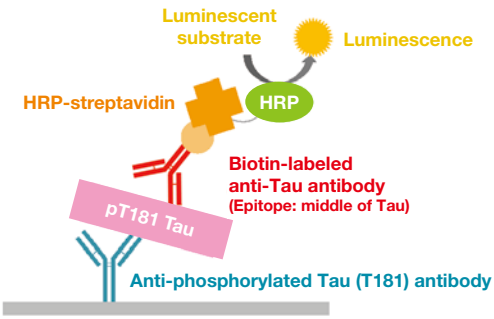
A higher correlation between tau concentration and MMSE scores was observed compared to the results obtained using competitive product.

Product Number	Product Name	Grade	Package Size
296-80401	Tau ELISA Kit *Wako*	for Immunochemistry	96 tests

Phosphorylated Tau (pT181)

Phosphorylated Tau T181 ELISA Kit *Wako* can be used for simple measurement of tau phosphorylated at threonine 181 (pT181). Like Tau ELISA Kit *Wako*, small amounts of samples (20 µL or more of human cerebrospinal fluid) can be used and provides high sensitivity.

Analyte	Phosphorylated Tau (pT181)
Calibration curve range	4.40-500 pg/mL
Analysis sample	Human Cerebrospinal fluid (Not suitable for serum and plasma)
Sample volume	20 µL
Measurement duration	20 hours
Detection method	Luminescence (a plate reader for luminescence measurement is required)



Product Number	Product Name	Grade	Package Size
298-81701	Phosphorylated Tau T181 ELISA Kit *Wako*	for Immunochemistry	96 tests

Lipopolysaccharide (LPS)

Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria. LPS is recognized by pattern recognition receptors (PRRs) and induces an innate immune response. LPS is known to induce activation of microglia to M1 type[1)] and is often used in microglial research.

1) Orihuela, R., McPherson, C. A., & Harry, G. J.: *Br. J. Pharmacol.*, **173**(4), 649(2016).

Product Number	Product Name	Package Size
124-05151	Lipopolysaccharide, from *E. coli* O127 (by phenol extraction)	25 mg
121-05161	Lipopolysaccharide, from *E. coli* O26 (by ultracentrifugation)	5 mg
128-05171	Lipopolysaccharide, from *E. coli* O55 (by ultracentrifugation)	5 mg
125-05181	Lipopolysaccharide, from *E. coli* O111 (by ultracentrifugation)	5 mg
124-06251	Lipopolysaccharide, from *E. coli* O113 (by ultracentrifugation)	5 mg
122-05191	Lipopolysaccharide, from *E. coli* O127 (by ultracentrifugation)	5 mg
120-06471	Lipopolysaccharide, from *E. coli* O128 (by ultracentrifugation)	5 mg
129-05461	Lipopolysaccharide, from *E. coli* O157 (by ultracentrifugation)	5 mg
126-05971	Lipopolysaccharide, from *Salmonella typhimurium* (by ultracentrifugation)	5 mg
124-05651	Lipopolysaccharide, from *Salmonella minnesota* 1114 (by ultracentrifugation)	5 mg
121-05661	Lipopolysaccharide, from *Salmonella minnesota* R595 (Re mutant) (by ultracentrifugation)	5 mg
126-06331	Lipopolysaccharide, from *Bordetella pertussis* Tohama (by ultracentrifugation)	2 mg
129-05961	Lipopolysaccharide, from *Pseudomonas aeruginosa* PAO1 (by ultracentrifugation)	5 mg

Microglia Activation Inhibitors

Antibiotics

Minocycline, a tetracycline antibiotic, inhibits microglial activation.

Product Number	Product Name	Package Size
135-18671	Minocycline Hydrochloride	200 mg
131-18673		1 g

Polyphenol

Resveratrol, a polyphenol with antioxidant activity, is found in red wine and grapes. It inhibits microglial activation[1)].

1) Zhang, F., Liu, J., and Shi, J. S.: *Eur. J. Pharmacol.*, **636**(1-3), 1(2010).

Product Number	Product Name	Package Size
184-02771	Resveratrol, Synthetic	1 g
180-02773		5 g
182-02772		25 g

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Microglia activated to M1 type by LPS, and other compounds release pro-inflammatory cytokines and promote inflammatory responses. Non-steroidal anti-inflammatory drugs (NSAIDs) suppress the release of pro-inflammatory cytokines.

Product Number	Product Name	Package Size
098-02641	Ibuprofen	1 g
094-02643		10 g
093-02473	Indomethacin	5 g
097-02471		10 g
095-02472		25 g
043-22851	Diclofenac Sodium	10 g

Product Number	Product Name	Package Size
191-03142	Sodium Salicylate	25 g
195-03145		500 g
015-10262	Acetylsalicylic Acid	25 g
017-10261		100 g
019-10265		500 g
186-03331	Rofecoxib	100 mg