

Anti-Iba1 Antibody [JM36-62]

ET1705-78



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	WB, IHC-P, IHC-Fr, IF-Tissue, IF-Cell, FC, IP, mIHC
Molecular Wt:	Predicted band size: 17 kDa
Clone number:	JM36-62

Description: Ionized calcium-binding adapter molecule 1 (Iba1), also known as allograft inflammatory factor-1 (AIF-1), is a 147 amino acid cytoplasmic, calcium-binding protein that is thought to play a role in macrophage activation and function. Iba1, containing two EF domains, is induced by cytokines and interferons. In an unstimulated state, Iba1 colocalizes with actin, and upon stimulation, translocates to lamellipodia. It is also a marker of human microglia and is expressed by macrophages in injured skeletal muscle. The gene encoding Iba1 maps to chromosome 6p21.33 and resides in the tumor necrosis factor (TNF) cluster of genes located in the region represented by the human major histocompatibility complex (MHC).

Immunogen: Synthetic peptide within N-terminal human Iba1.

Positive control: THP-1 cell lysate, mouse spleen tissue lysate, rat spleen tissue lysate, THP-1, J774A.1, RAW264.7, C6, mouse hippocampus tissue, human kidney tissue, human spleen tissue, mouse brain tissue, rat brain tissue.

Subcellular location: Cytoplasm, cytoskeleton, Cell projection, ruffle membrane, Cell projection, phagocytic cup.

Database links: SwissProt: P55008 Human | O70200 Mouse | P55009 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:1,000
IHC-Fr	1:1,000-1:2,000
IF-Tissue	1:500
IF-Cell	1:250-1:500
FC	1:1,000
IP	Use at an assay dependent concentration.
mIHC	1:2,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

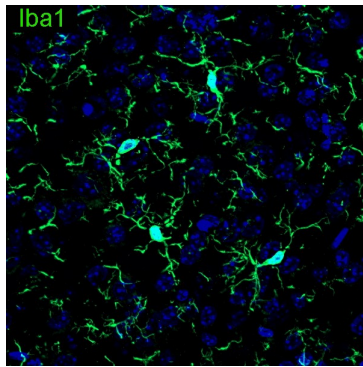
Technical:0086-571-89986345

Service mail:support@huabio.cn

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

**Fig1:** Application: IHC-Fr

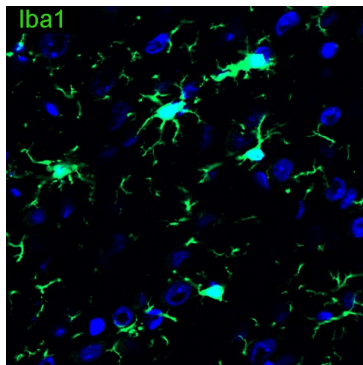
Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/2,000

Antigen retrieval: Not required

**Fig2:** Application: IHC-Fr

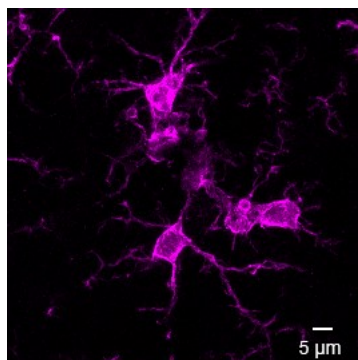
Species: Rat

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/1,000

Antigen retrieval: Not required

**Fig3:** Application: IHC-Fr

Species: Mouse

Site: Hippocampus (APP-PS1 mouse)

Sample: Frozen section

Antibody concentration: 1/500

Date by courtesy of: Mr. Chenxin Ma, School of Basic Medical Sciences, Zhejiang University

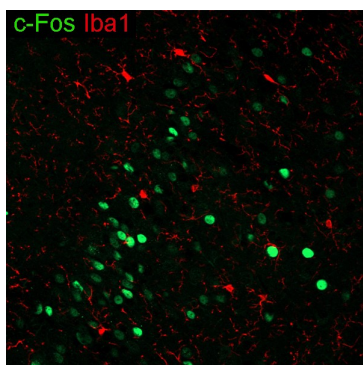
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**Fig4:** Application: IHC-Fr

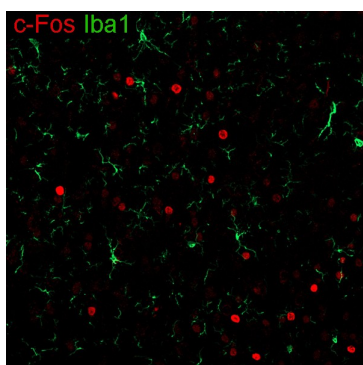
Species: Mouse

Site: Cerebral cortex (restraint stress induced)

Sample: Frozen section

Antibody concentration: 1/1,000 (Iba1, ET1705-78, Rabbit, red); 1/5,000 (c-Fos, HA601373, Rat, green)

Antigen retrieval: Not required

**Fig5:** Application: IHC-Fr

Species: Rat

Site: Cerebral cortex (restraint stress induced)

Sample: Frozen section

Antibody concentration: 1/1,000 (Iba1, ET1705-78, Rabbit, green); 1/1,000 (c-Fos, HA601373, Mouse, red)

Antigen retrieval: Not required

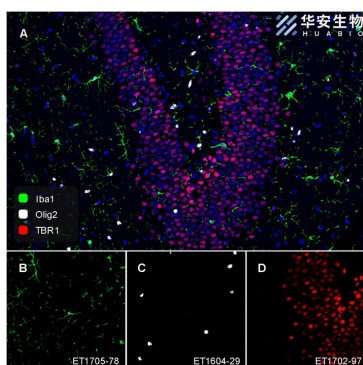


Fig6: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Iba1 (ET1705-78, Green), anti-Olig2 (ET1604-29, White) and anti-TBR1 (ET1702-97, Red) on brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1705-78 (1/2,000 dilution), ET1604-29 (1/1,000 dilution) and ET1702-97 (1/1,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

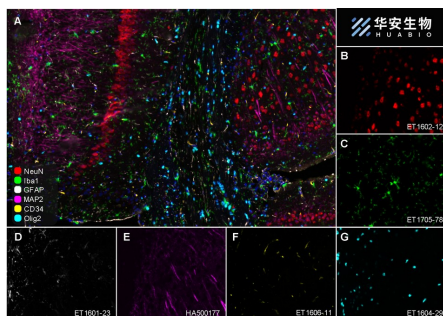
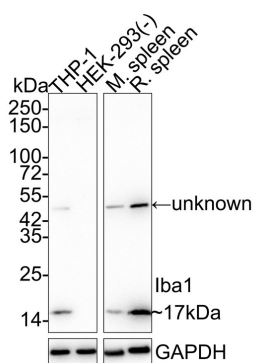


Fig7: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-Iba1 (ET1705-78, green), anti-GFAP (ET1601-23, gray), anti-Olig2 (ET1604-29, cyan), anti-MAP2 (HA500177, magenta) and anti-CD34 (ET1606-11, yellow) on mouse brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in six rounds of staining: in the order of ET1602-12 (1/5,000 dilution), ET1705-78 (1/2,000 dilution), ET1601-23 (1/5,000 dilution), ET1604-29 (1/1,000 dilution), HA500177 (1/5,000 dilution) and ET1606-11 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Fig8: Western blot analysis of Iba1 on different lysates with Rabbit anti-Iba1 antibody (ET1705-78) at 1/5,000 dilution.

Lane 1: THP-1 cell lysate
 Lane 2: HEK-293 cell lysate (negative)
 Lane 3: Mouse spleen tissue lysate
 Lane 4: Rat spleen tissue lysate



Lysates/proteins at 20 µg/Lane.

Predicted band size: 17 kDa
 Observed band size: 17 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1705-78) at 1/5,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

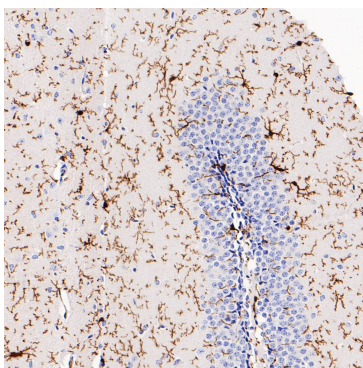


Fig9: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-78) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

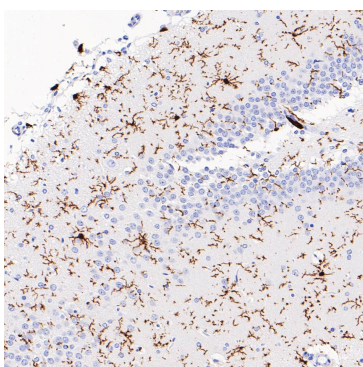


Fig10: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-78) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

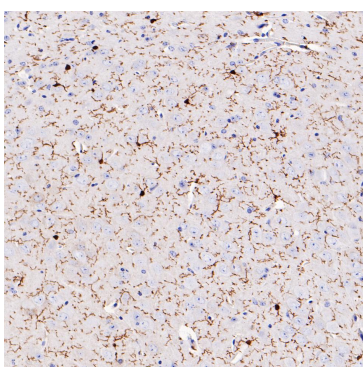


Fig11: Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-78) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

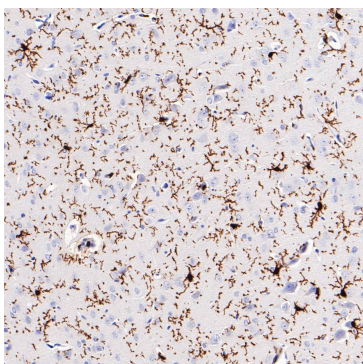


Fig12: Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-78) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

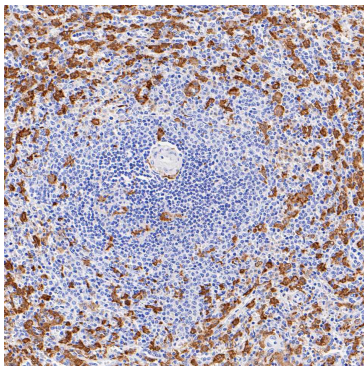


Fig13: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-78) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

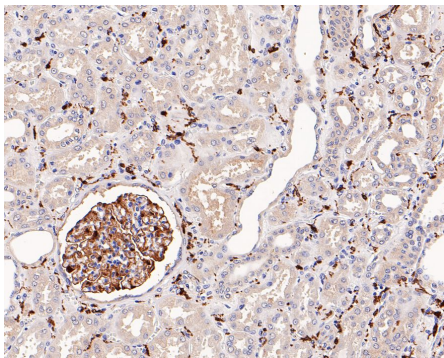


Fig14: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1705-78) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

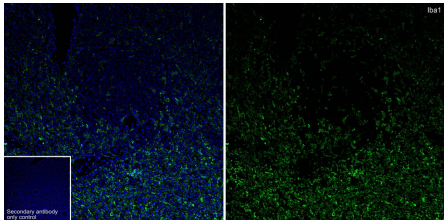


Fig15: Application: IF-tissue

Species: Human

Site: Spleen

Sample: Paraffin-embedded section

Antibody concentration: 1/500

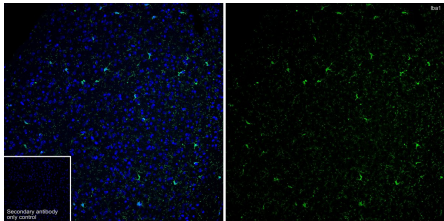


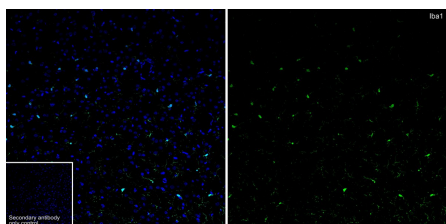
Fig16: Application: IF-tissue

Species: Mouse

Site: Cerebral cortex

Sample: Paraffin-embedded section

Antibody concentration: 1/500

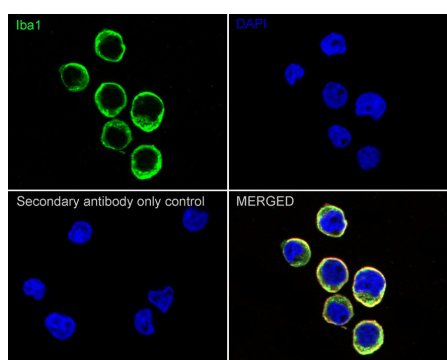
**Fig17:** Application: IF-tissue

Species: Rat

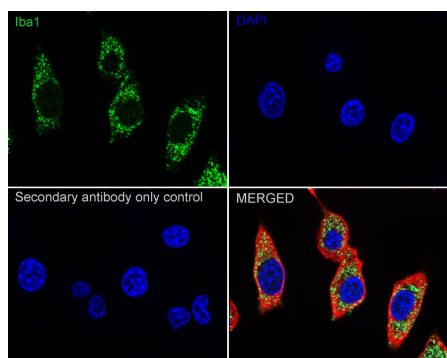
Site: Cerebral cortex

Sample: Paraffin-embedded section

Antibody concentration: 1/500

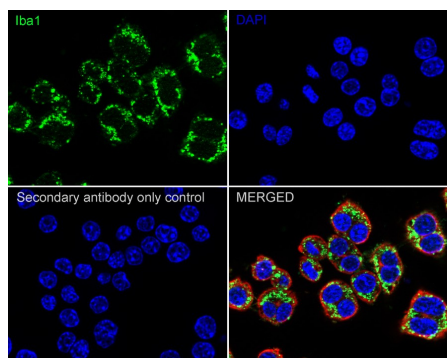
Fig18: Immunocytochemistry analysis of THP-1 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig19: Immunocytochemistry analysis of BV2 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/500 dilution.

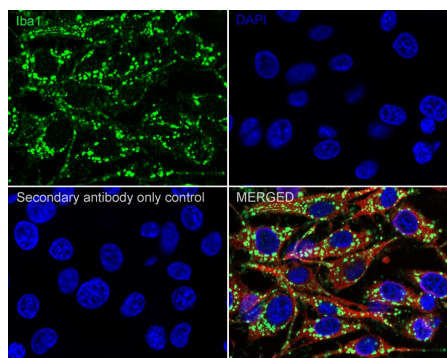
Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Iba1 antibody (ET1705-78) at 1/500 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig20: Immunocytochemistry analysis of RAW264.7 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig21: Immunocytochemistry analysis of C6 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

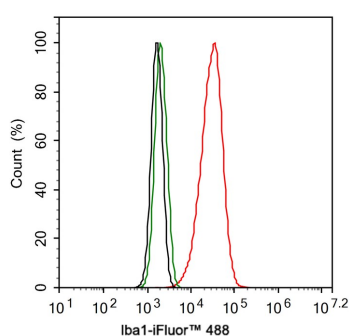


Fig22: Flow cytometric analysis of THP-1 cells labeling Iba1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-78, 1 µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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superimposed upon progressive neurodegeneration. Brain Behav Immun 39:233-244 (2017).

2. Arentsen T et al. The bacterial peptidoglycan-sensing molecule Pglyrp2 modulates brain development and behavior.

