Anti-Iba1 Antibody [JM36-62]

ET1705-78

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IP, IF-Cell, IF-Tissue, IHC-Fr, 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
IF-Cell	1:100-1:250
IF-Tissue	1:50-1:100
IHC-P	1:1,000
IHC-Fr	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: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

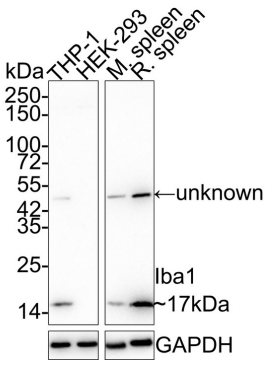

Fig1: Western blot analysis of Iba1 on different lysates with Rabbit anti-Iba1 antibody (ET1705-78) at 1/5,000 dilution.

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℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

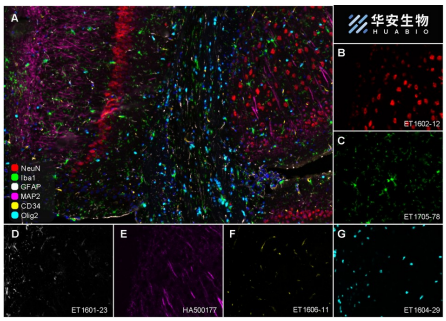

Fig2: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-Iba1 (ET1705-78, green), anti-GFAP (ET1601-23, gray), anti-Olig2 (ET1604-29, cyan), anti-MAP2 (HA500177, magenta) and 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℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

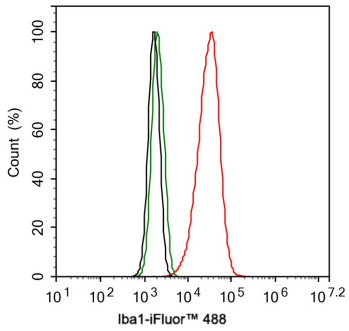

Fig3: Flow cytometric analysis of THP-1 cells labeling Iba1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-78, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

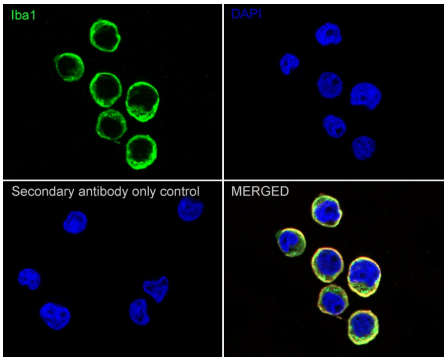

Fig4: Immunocytochemistry analysis of THP-1 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution.

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 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

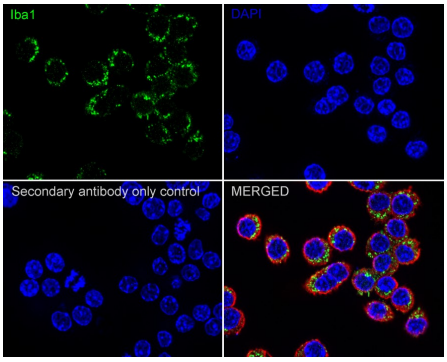

Fig5: Immunocytochemistry analysis of J774A.1 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution.

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 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

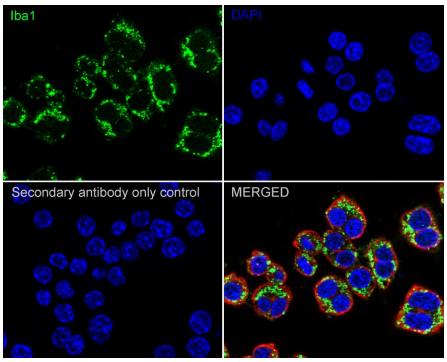

Fig6: Immunocytochemistry analysis of RAW264.7 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution.

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 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

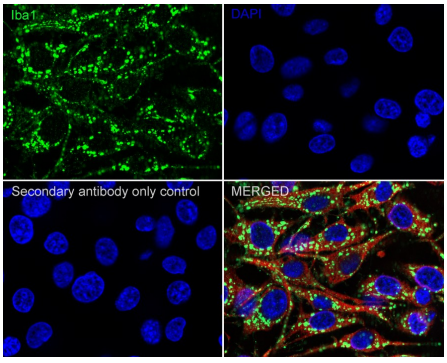

Fig7: Immunocytochemistry analysis of C6 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/100 dilution.

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 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

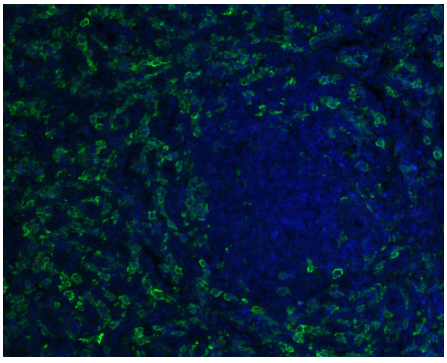

Fig8: Immunofluorescence staining of paraffin-embedded human spleen tissue using anti-Iba1 antibody.The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with ET1705-78 at 1/50 dilution for 10 hours at 4℃ and detected using Alexa Fluor® 488 conjugate-Goat anti-Rabbit IgG (H+L) Secondary Antibody at a dilution of 1:500 for 1 hour at room temperature.

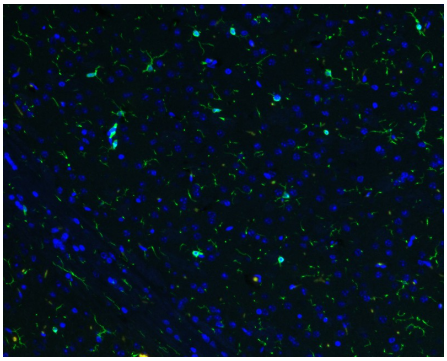

Fig9: Immunofluorescence staining of paraffin-embedded mouse brain tissue using anti-Iba1 antibody.The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with ET1705-78 at 1/50 dilution for 10 hours at 4℃ and detected using Alexa Fluor® 488 conjugate-Goat anti-Rabbit IgG (H+L) Secondary Antibody at a dilution of 1:500 for 1 hour at room temperature.

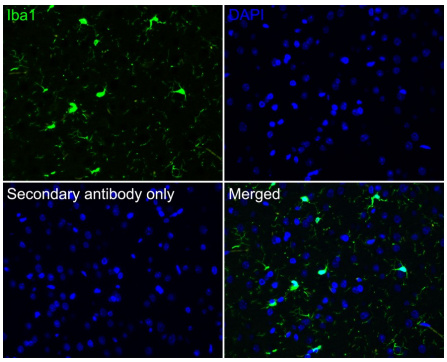

Fig10: Immunofluorescence analysis of paraffin-embedded rat brain tissue labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1705-78, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

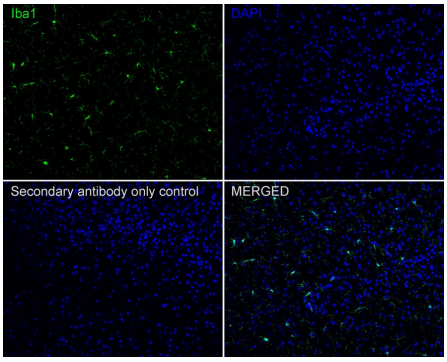

Fig11: Immunofluorescence analysis of frozen mouse brain tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1705-78, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

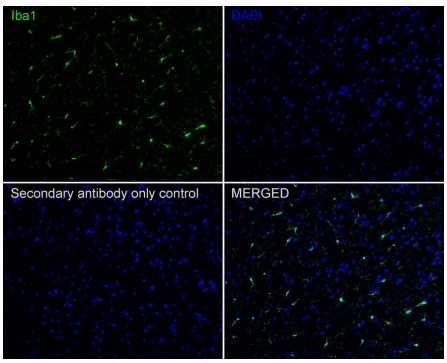

Fig12: Immunofluorescence analysis of frozen rat brain tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1705-78, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

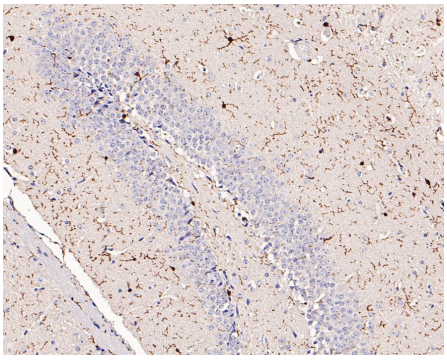

Fig13: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

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 ddH_2O 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.

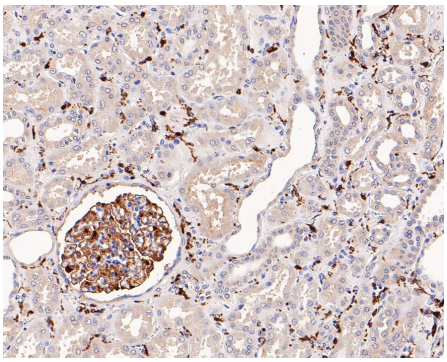

Fig14: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

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 ddH_2O 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.

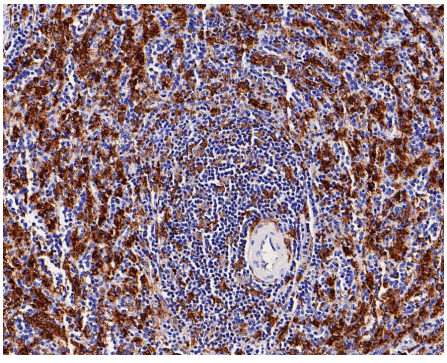

Fig15: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

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 ddH_2O 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.

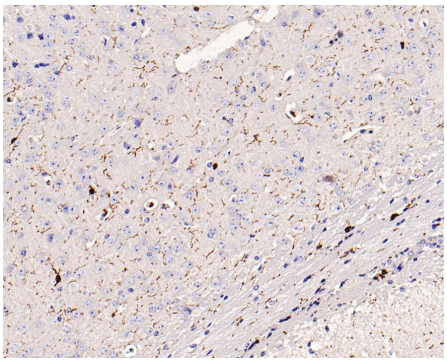

Fig16: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

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 ddH_2O 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.

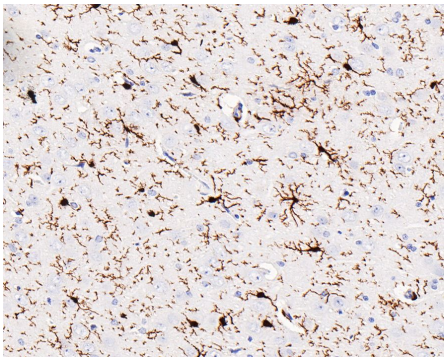

Fig17: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

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 ddH_2O 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.

Background References

1. Hennessy E et al. Systemic TNF-a produces acute cognitive dysfunction and exaggerated sickness behavior when superimposed upon progressive neurodegeneration. Brain Behav Immun 59:233-244 (2017).
2. Arentsen T et al. The bacterial peptidoglycan-sensing molecule Pglyrp2 modulates brain development and behavior. Mol Psychiatry 22:257-266 (2017).