abcam

Product datasheet

Anti-Iba1 antibody [EPR16588] - BSA and Azide free ab220815

Recombinant RabMAb

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Overview

Product name	Anti-Iba1 antibody [EPR16588] - BSA and Azide free
Description	Rabbit monoclonal [EPR16588] to Iba1 - BSA and Azide free
Host species	Rabbit
Specificity	For **ab178846** Abcam recommends blocking in 3% milk for cleanest results in WB. Blocking with BSA gives slightly higher background.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HL-60, THP-1, U937, RAW 264.7 and NR8383 whole cell lysates; Human, mouse and rat spleen lysates; Mouse testis and liver lysates; Rat and mouse hippocampus and brain tissue lysates. IHC-P: Human Cerebral cortex, human hippocampus; Rat and mouse normal brain tissues. Flow Cyt (intra): U937 cells, Raw264.7 ICC/IF: Rat hippocampal mixed glia.
General notes	ab220815 is the carrier-free version of **ab178846**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply

- Animal-free production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16588
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab220815 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

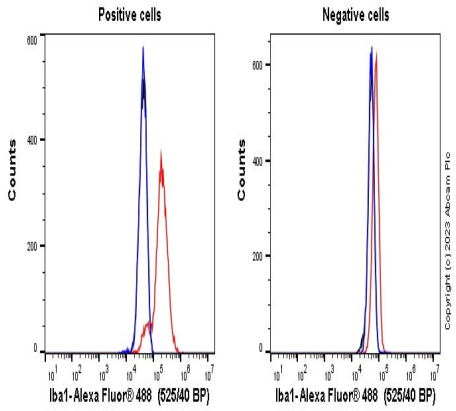
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 10, 15 kDa (predicted molecular weight: 17 kDa). Abcam recommends blocking in 3% milk for cleanest results in WB. Blocking with BSA gives slightly higher background.
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function	Actin-binding protein that enhances membrane ruffling and RAC activation. Enhances the actin-bundling activity of LCP1. Binds calcium. Plays a role in RAC signaling and in phagocytosis. May play a role in macrophage activation and function. Promotes the proliferation of vascular smooth muscle cells and of T-lymphocytes. Enhances lymphocyte migration. Plays a role in vascular inflammation.
Tissue specificity	Detected in T-lymphocytes and peripheral blood mononuclear cells.

Sequence similarities	Contains 2 EF-hand domains.
Post-translational modifications	Phosphorylated on serine residues.
Cellular localization	Cytoplasm > cytoskeleton. Cell projection > ruffle membrane. Associated with the actin cytoskeleton at membrane ruffles and at sites of phagocytosis.

Images



Flow Cytometry (Intracellular) - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

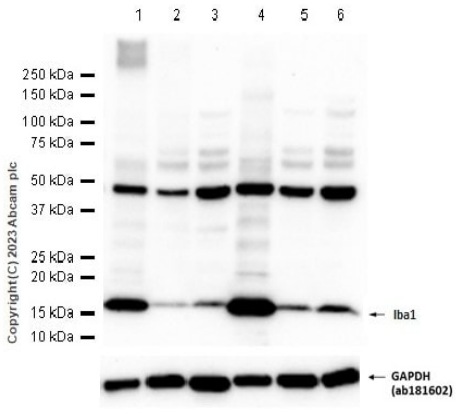
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).

Flow cytometry overlay histogram showing left Raw264.7 positive cells and right negative NIH3T3 stained with **ab178846** (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10µg/ml anti CD16/CD32 and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (**ab178846**) (1x 10^6 in 100µl at 0.2µg/ml (1/9850)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

All lanes : Anti-Iba1 antibody [EPR16588] (**ab178846**) at 1/1000 dilution

Lane 1 : Mouse spleen tissue lysate
Lane 2 : Mouse brain tissue lysate
Lane 3 : Mouse hippocampus tissue lysate
Lane 4 : Rat spleen tissue lysate
Lane 5 : Rat brain tissue lysate
Lane 6 : Rat hippocampus tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 40 seconds

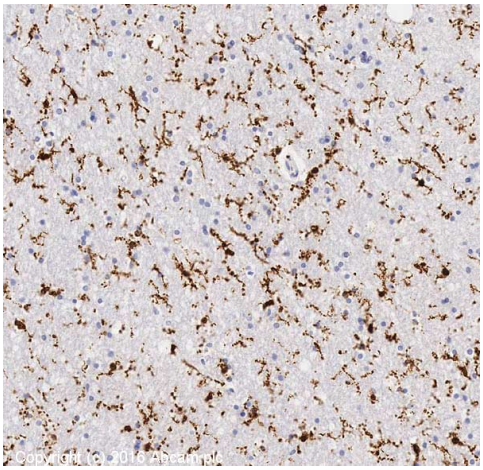
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

IBA1 is a relatively minor protein of brain and is much more abundant in spleen (PMID: 8912632, PMID: 29232670). We suggest loading higher amount of brain lysate or using lower dilution of antibody for detecting signal in brain related lysates.

ab181602 was used as loading control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).

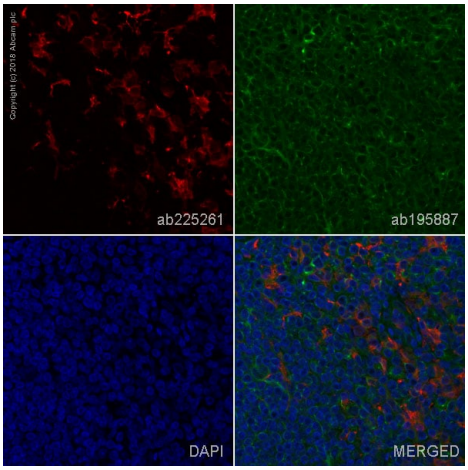


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

IHC image of Iba1 staining in human normal hippocampus formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with **ab178846**, 1/2000 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

Clone EPR16588 (ab220815) has been successfully conjugated by Abcam. This image was generated using Anti-Iba1 antibody [EPR16588] (Alexa Fluor® 647). Please refer to **ab225261** for protocol details.

IHC image of Iba1 staining in a section of formalin-fixed paraffin-embedded normal human tonsil*.

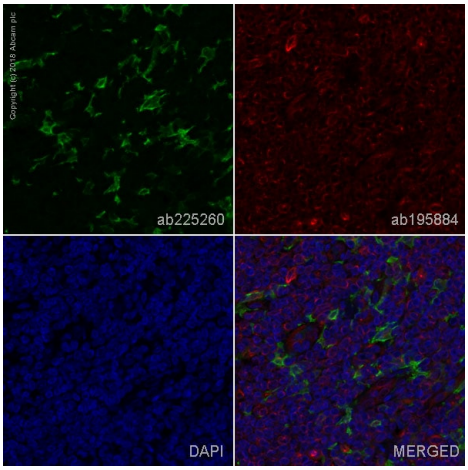
The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) in a Biocare Medical NxGen pressure cooker using retrieval settings of 110^{o}C for 20 minutes. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with **ab225261** at 1/100 dilution (shown in red) and counterstained using **ab195887**, Mouse monoclonal to

alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

Clone EPR16588 (ab220815) has been successfully conjugated by Abcam. This image was generated using Anti-Iba1 antibody [EPR16588] (Alexa Fluor® 488). Please refer to **ab225260** for protocol details.

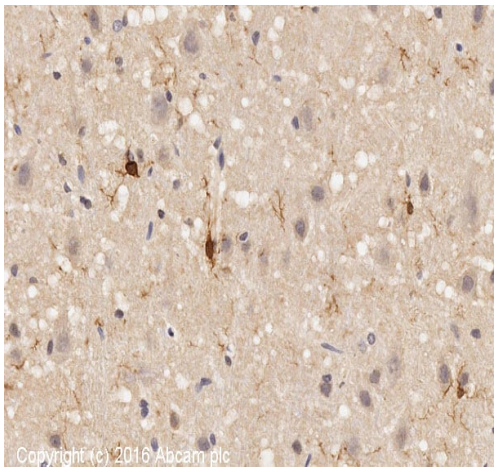
IHC image of Iba1 staining in a section of formalin-fixed paraffin-embedded normal human tonsil*.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) in a Biocare Medical NxGen pressure cooker using retrieval settings of 110^{O}C for 20 minutes. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with **ab225260** at 1/100 dilution (shown in green) and counterstained using **ab195884**, Rat monoclonal to Tubulin (Alexa Fluor® 647), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.

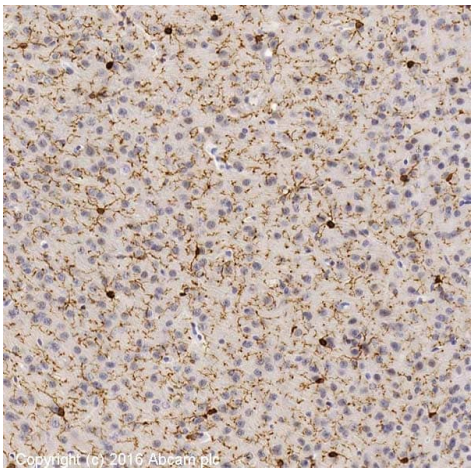


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

IHC image of Iba1 staining in rat normal brain formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with **ab178846**, 1/2000 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).

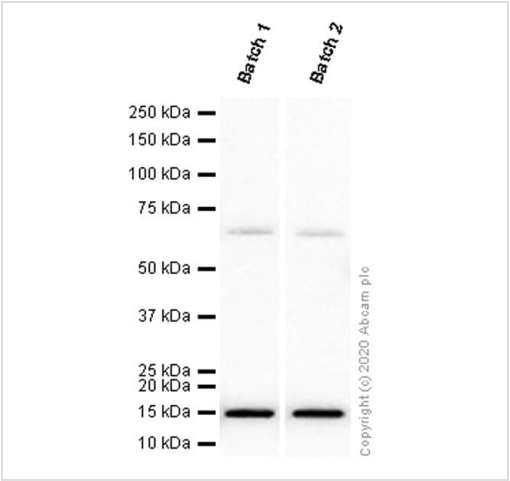


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

IHC image of Iba1 staining in mouse normal brain formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with **ab178846**, 1/2000 dilution, for 15 mins at room temperature. A goat anti-rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

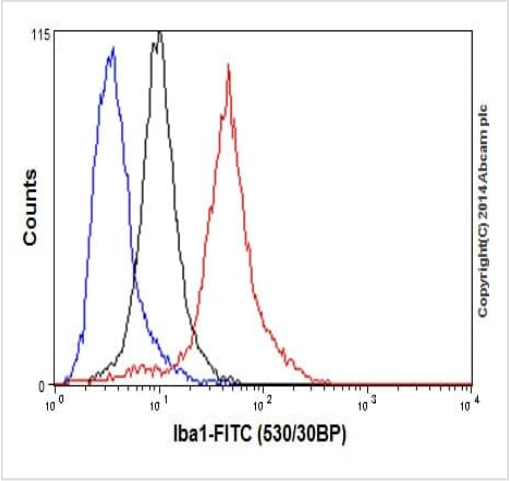
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).



Western blot - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

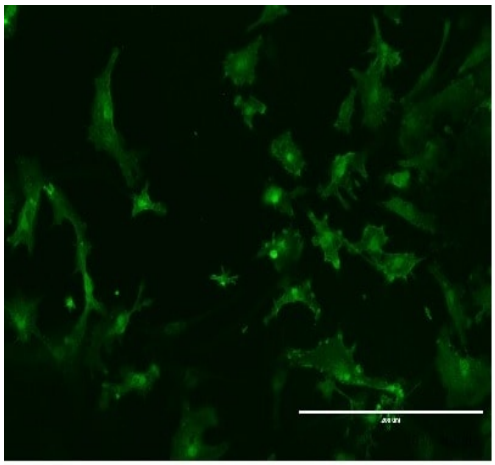
This data was developed using **ab178846**, the same antibody clone in a different buffer formulation. Different batches of **ab178846** were tested on THP-1 (Human monocytic leukemia monocyte) lysate at 0.02 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 15 kDa.



Flow Cytometry (Intracellular) - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

Intracellular Flow Cytometry analysis of 2% paraformaldehyde fixed U937 (human histiocytic lymphoma cell line) cells labeling Iba1 with **ab178846** at 1/160 dilution (red line). Secondary antibody used is a goat anti rabbit IgG (FITC) at 1/150 dilution. The isotype control is rabbit monoclonal IgG (black line). The unlabeled control is cells without incubation with primary and secondary antibodies (blue line).

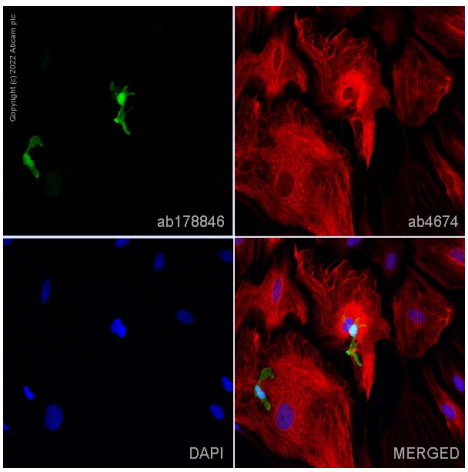
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).



Immunocytochemistry/ Immunofluorescence - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

0.1% Triton-X 100 permeabilized paraformaldehyde-fixed Mouse cell Microglia cells labeling Iba1 (green) using **ab178846** at 1/500 dilution in ICC/IF analysis.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).

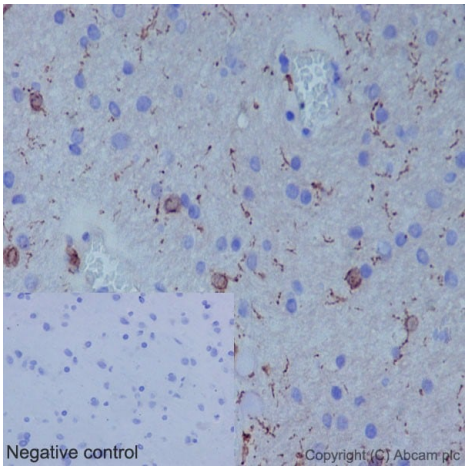


Immunocytochemistry/ Immunofluorescence - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

Immunofluorescence staining of Iba-1 using **ab178846** in primary rat hippocampal mixed glia, (prepared from P2 rat hippocampal brain area, obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. SDPHP4m), DIV4. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton-X-100 (in PBS) for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab178846** at 0.1 µg/ml and **ab4674**, Anti-GFAP antibody, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150176**, Goat Anti-Chicken IgY H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Images were acquired with the Perkin Elmer Operetta HCA and a maximum intensity projection of confocal sections is shown. The antibody **ab178846** gave comparable results using MeOH fixation (100%, 5 min).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).

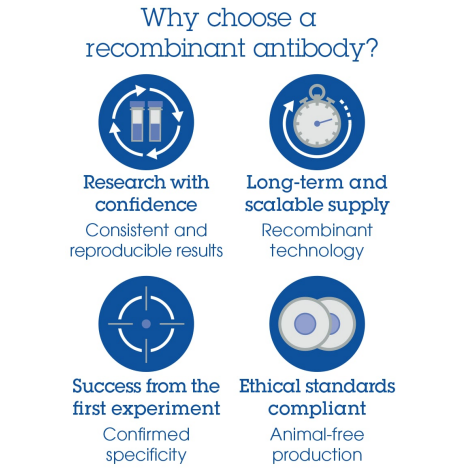


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

Immunohistochemical analysis of paraffin-embedded Human cerebral cortex tissue labeling Iba1 with **ab178846** at a 1/2000 dilution showing cytoplasm and nuclear staining on Glial cells. Counter stained with hematoxylin. Prediluted HRP Polymer for Rabbit/Mouse IgG was used as the secondary aantibody. Negative control also shown.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab178846**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-Iba1 antibody [EPR16588] - BSA and Azide free (ab220815)

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