abcam

Product datasheet

Anti-Iba1 antibody [EPR16588] ab178846

重组 **RabMAb**

★★★★★ **45 Abreviews**   **285 References**   19 图像

概述

产品名称	Anti-Iba1抗体[EPR16588]
描述	兔单克隆抗体[EPR16588] to Iba1
宿主	Rabbit
特异性	For ab178846 Abcam recommends blocking in 3% milk for cleanest results in WB. Blocking with BSA gives slightly higher background. 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
经测试应用	**适用于:** Flow Cyt (Intra), IHC-P, WB, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

纯度	Protein A purified
克隆	单克隆
克隆编号	EPR16588
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab178846于以下的经测试应用**

"应用说明"部分 下显示的仅为**推荐的起始稀释度**;实际**最佳**的稀释度/浓度应**由**使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/160.
IHC-P	★★★★★ (18)	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ (2)	1/500 - 1/2000. 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.
ICC/IF	★★★★★ (6)	Use a concentration of 0.1 - 1 µg/ml.

靶标

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功能	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.
组织特异性	Detected in T-lymphocytes and peripheral blood mononuclear cells.
序列相似性	Contains 2 EF-hand domains.
翻译后修饰	Phosphorylated on serine residues.
细胞定位	Cytoplasm > cytoskeleton. Cell projection > ruffle membrane. Associated with the actin cytoskeleton at membrane ruffles and at sites of phagocytosis.

图片
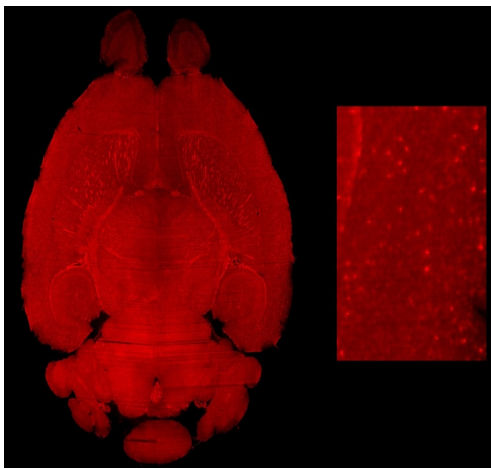
Immunohistochemistry - Anti-Iba1 antibody [EPR16588] (ab178846)

Anti-Iba1 ab178846 was used with Tissue clearing kit – CUBIC (**ab316246**) and 3D Tissue Staining Kit – CUBIC (**ab316248**) to penetrate, stain and clear a whole mouse brain.

Learn more about **tissue clearing kits, reagents, and protocols** designed to make it easier to stain whole brains and get more data from each valuable tissue sample.

For a whole mouse brain, we recommend starting with 3.5 ug of ab178846 and using a Fab fragment secondary antibody with 2.34 μg to create an antibody complex before 3D staining (see protocol for details). Additive A was used during the staining process.

The sample was imaged using a light-sheet microscope**.**
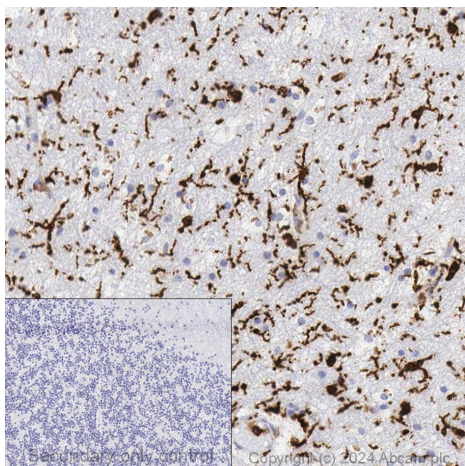
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] (ab178846)

Immunohistochemical analysis of formalin fixed paraffin embedded human cerebellum labelling Iba1 with ab178846 at a dilution of 1/4000. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an ChromoMap DAB (RUO) IHC Detection Kit with anti rabbit HQ and anti HQ HRP. Heat mediated antigen retrieval was conducted for 24 min with DISCOVERY cell conditioning solution (CC1) 100°C, pH 8.5. ab178846 was incubated at 37°C for 16 min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.
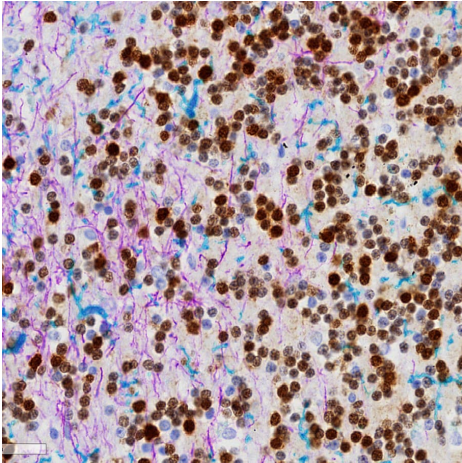
Multiplex immunohistochemistry - Anti-Iba1 antibody [EPR16588] (ab178846)

Chromogenic multiplex immunohistochemical staining of FFPE normal human cerebellum tissue. **ab177487**, anti-NeuN DAB chromogen. Ab68428, anti-GFAP purple chromogen and ab178846, anti- Iba1 teal chromogen plus haematoxylin counterstain.

Chromogenic immunostaining was performed on a Roche Ventana Discovery Ultra instrument. The section was deparaffinised and incubated with CC1 solution for 24min 100°C. Following this with 3 rounds of staining in the order of **ab177487** (1/600), ab178846 (1/4000) **ab68428** (1/1000). Between rounds of staining, antibody denaturation was conducted using Ultra CC2 solution for 8min at 100°C to avoid cross reactivity. Signal was developed with anti-rabbit HQ followed by anti-HQ HRP coupled with Chromomap DAB kit, Discovery purple or Discovery teal chromogens and haematoxylin II counterstain.
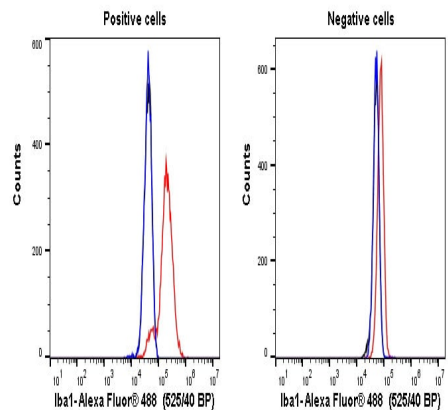

Flow Cytometry (Intracellular) - Anti-Iba1 antibody [EPR16588] (ab178846)

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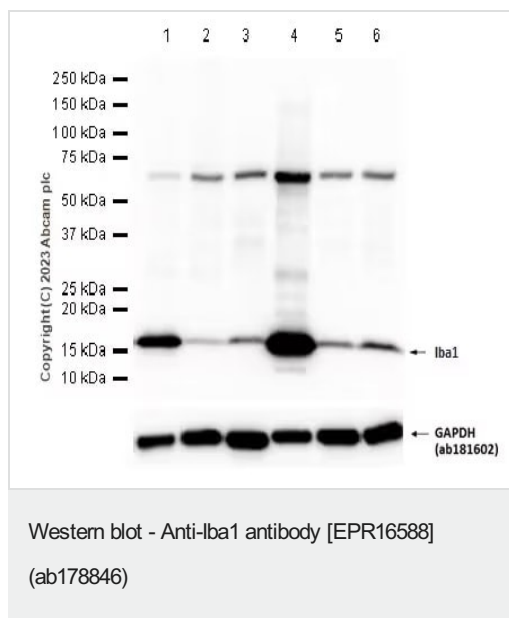
All lanes : Anti-Iba1 antibody [EPR16588] (ab178846) at 1/1000 dilution

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.
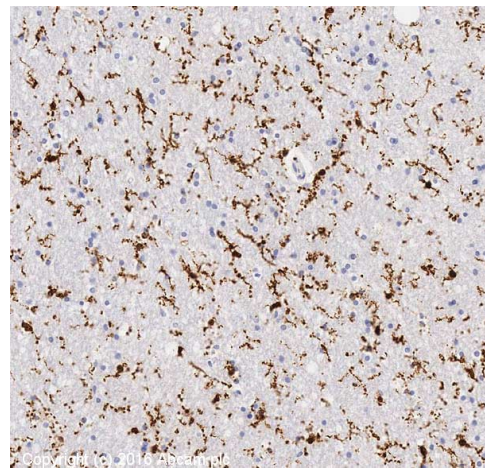
ab181602 was used as loading control.
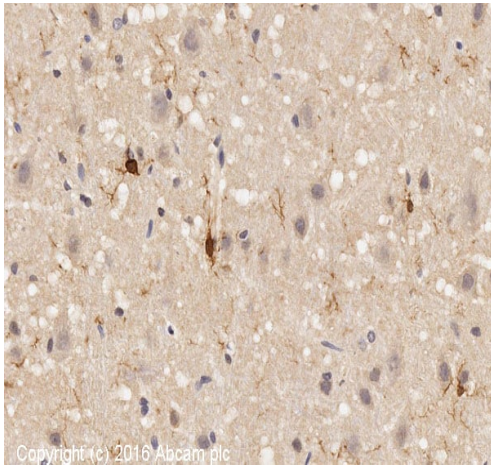
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] (ab178846)

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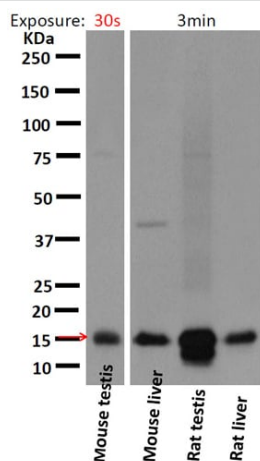
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] (ab178846)

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.

Western blot - Anti-Iba1 antibody [EPR16588]
(ab178846)

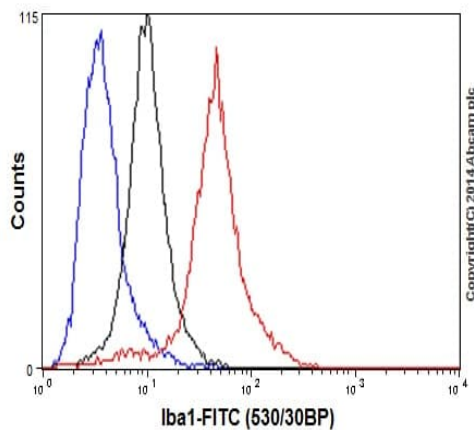**All lanes :** Anti-Iba1 antibody [EPR16588] (ab178846)

Lane 1 : Mouse testis
Lane 2 : Mouse liver
**Lane 3 :** Rat testis
**Lane 4 :** Rat liver

Predicted band size: 17 kDa

Flow Cytometry (Intracellular) - Anti-Iba1 antibody
[EPR16588] (ab178846)

Intracellular Flow Cytometry analysis of2% paraformaldehyde fixed
U937 (human histiocytic lymphoma cell line) cells labeling Iba1with
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).
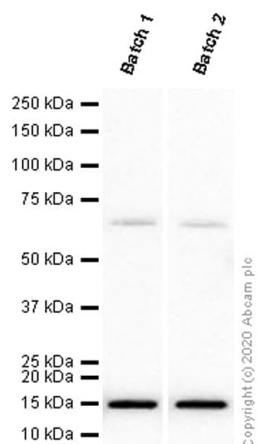
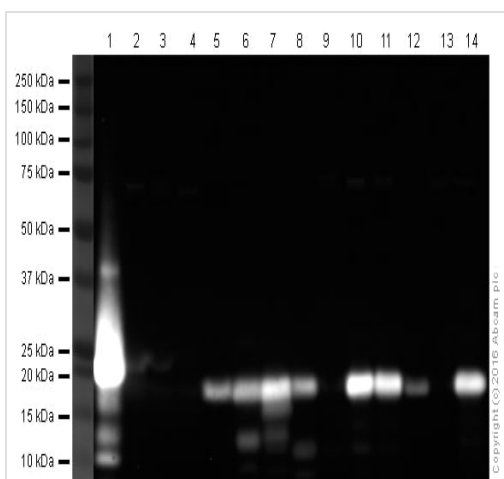Western blot - Anti-Iba1 antibody [EPR16588]
(ab178846)

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.
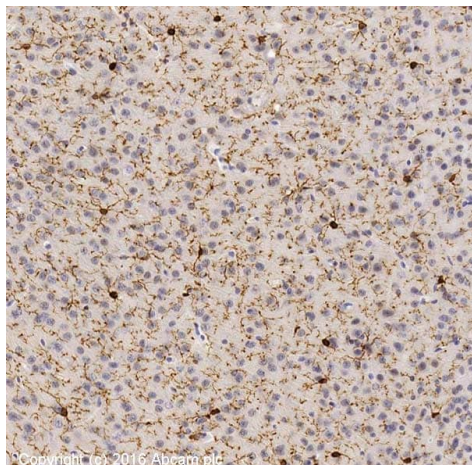
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] (ab178846)

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.

Western blot - Anti-Iba1 antibody [EPR16588] (ab178846)

All lanes : Anti-Iba1 antibody [EPR16588] (ab178846) at 1/500 dilution

Lane 1 : Human Iba1 recombinant protein at 0.1 µg

Lane 2 : HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate at 20 µg

Lane 3 : A431 (human epidermoid carcinoma cell line) whole cell lysate at 20 µg

Lane 4 : NIH/3T3 (mouse embyro fibroblast cell line) whole cell lysate at 30 µg

Lane 5 : Human spleen tissue lysate at 20 µg

Lane 6 : Mouse spleen tissue lysate at 30 µg

Lane 7 : Rat spleen tissue lysate at 30 µg

Lane 8 : U937 (human histiocytic lymphoma cell line) whole cell lysate at 30 µg

Lane 9 : MOLT-4 (human lymphoblastic leukemia cell line) whole cell lysate at 20 µg

Lane 10 : THP-1 (human monocytic leukemia cell line) whole cell lysate at 30 µg

Lane 11 : THP-1 whole cell lysate, PMA treated at 30 µg

Lane 12 : RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 30 µg

Lane 13 : C6 (rat glial tumor cell line) whole cell lysate at 30 µg

Lane 14 : NR8383 whole cell lysate at 30 µg

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 17 kDa

Exposure time: 1 minute

Abcam recommends blocking in milk for cleaner blots with reduced background, in comparison to BSA.

This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with ab178846 (anti-Iba1 antibody; 1/500 dilution) for 18 hours at 4°C. Antibody binding was detected using **ab97040** (HRP-labelled goat anti-mouse IgG) at 1:50,000 dilution for 1 hour at room temperature and visualised using ECL development solution **ab133406**.
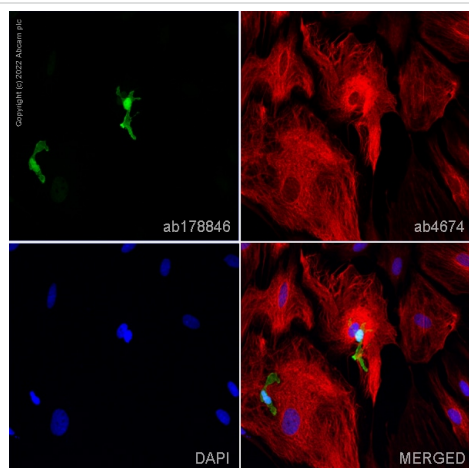
Immunocytochemistry/ Immunofluorescence - Anti-Iba1 antibody [EPR16588] (ab178846)

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).
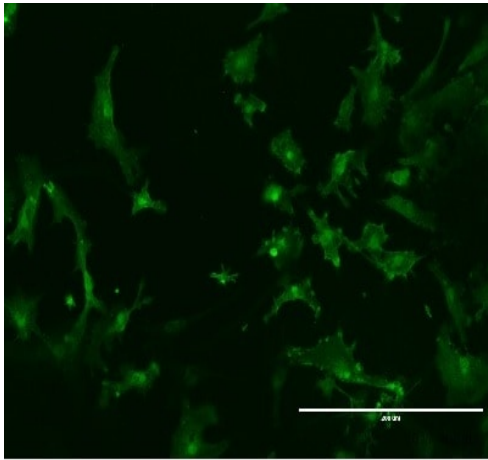
0.1% Triton-X 100 permeabilized paraformaldehyde-fixed Mouse cell Microglia cells labeling Iba1 (green) using ab178846 at 1/500 dilution in ICC/IF analysis.
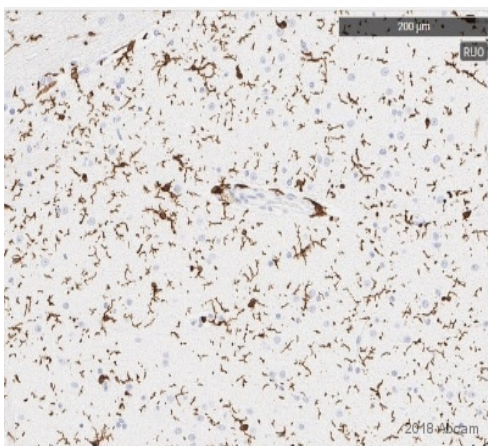
Immunocytochemistry/ Immunofluorescence - Anti-Iba1 antibody [EPR16588] (ab178846)

Formaldehyde-fixed, paraffin-embedded cynomolgus monkey brain tissue stained for Iba1 using ab178846 at 1/6000 dilution in immunohistochemical analysis.

Antigen Retrieval: Heat mediated - Buffer/Enzyme Used: pH 9.0 EDTA

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] (ab178846)

This image was courtesy of an annonymous Abreview

Western blot - Anti-Iba1 antibody [EPR16588] (ab178846)
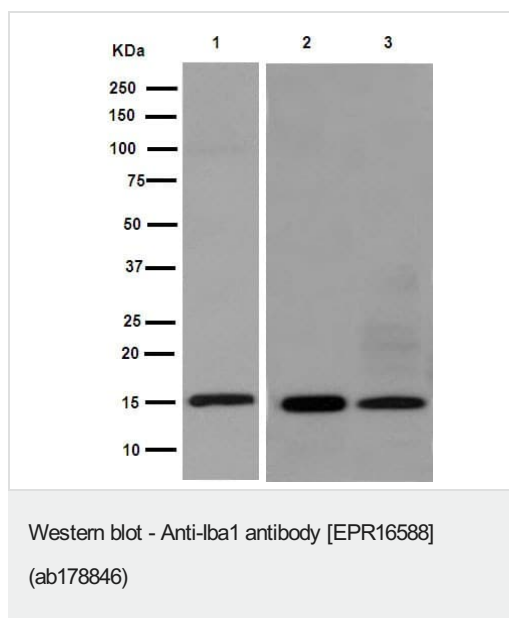
All lanes : Anti-Iba1 antibody [EPR16588] (ab178846) at 1/10000 dilution

Lane 1 : THP-1 (human monocytic leukemia cell line) whole cell lysate

Lane 2 : U937 (human histiocytic lymphoma cell line) whole cell lysate

Lane 3 : Human spleen whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution
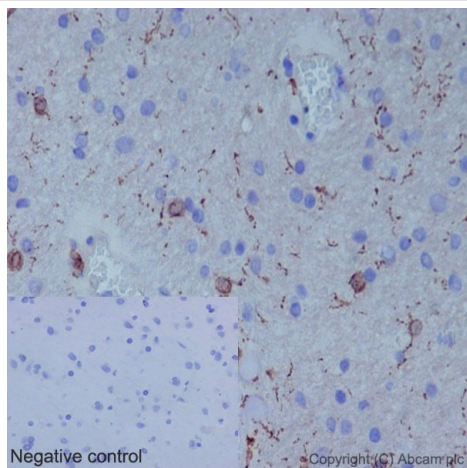
Developed using the ECL technique.

Predicted band size: 17 kDa

Observed band size: 15 kDa

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM /TBST

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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] (ab178846)
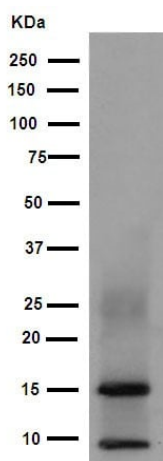
Anti-Iba1 antibody [EPR16588] (ab178846) at 1/2000 dilution + HL-60 (human promyelocytic leukemia cell line) whole cell lysate at 10 µg

Secondary
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 17 kDa
Observed band size: 10, 15 kDa

Western blot - Anti-Iba1 antibody [EPR16588] (ab178846)

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration:

5% NFDM /TBST.

Based on sequence analysis, ab178846 recognizes 2 isoforms with the predicted MWs of 17KDa and 11KDa, respectively.

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

Anti-Iba1 antibody [EPR16588] (ab178846)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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