abcam

Product datasheet

Anti-Iba1 antibody [EPR16588] ab178846

リコンビナント RabMAb

45 Abreviews 285 References 画像数 19

製品の概要

製品名	Anti-Iba1 antibody [EPR16588]
製品の詳細	Rabbit monoclonal [EPR16588] to Iba1
由来種	Rabbit
特異性	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
アプリケーション	**適用あり:** Flow Cyt (Intra), IHC-P, WB, ICC/IF
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	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.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR16588
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab178846の使用に適用されます

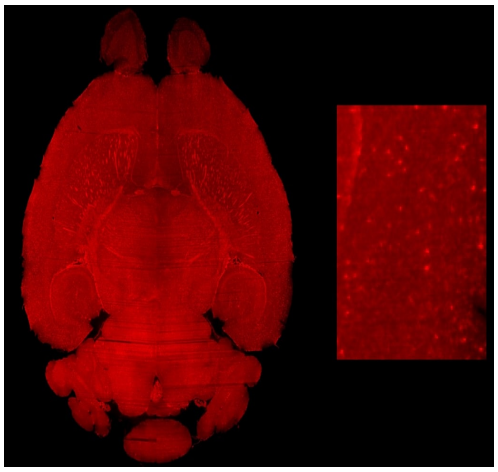
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

ターゲット情報

機能	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.
組織特異性	Detected in T-lymphocytes and peripheral blood mononuclear cells.
配列類似性	Contains 2 EF-hand domains.
翻訳後修飾	Phosphorylated on serine residues.
細胞内局在	Cytoplasm > cytoskeleton. Cell projection > ruffle membrane. Associated with the actin cytoskeleton at membrane ruffles and at sites of phagocytosis.

画像



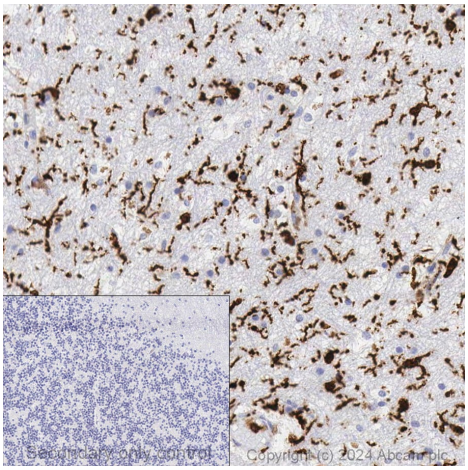
Immunohistochemistry - Anti-Iba1 antibody [EPR16588] (ab178846)

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.

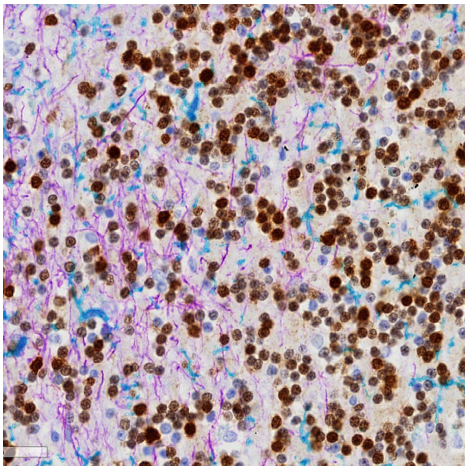
For a whole mouse brain, we recommend starting with 3.5 ug of ab178846 and using a Fab fragment secondary antibody with 2.34 µg to create an antibody complex before 3D staining (see protocol for details). Additive A was used during the staining process.

The sample was imaged using a light-sheet microscope**.**



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

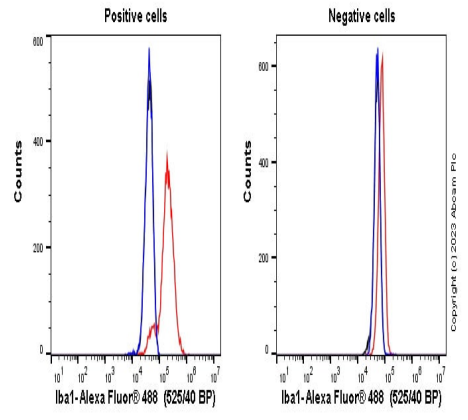
Immunohistochemical analysis of formalin fixed paraffin embedded human cerebellum labelling Iba1 with ab178846 at a dilution of 1/4000. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an ChromoMap DAB (RUO) IHC Detection Kit with anti rabbit HQ 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.



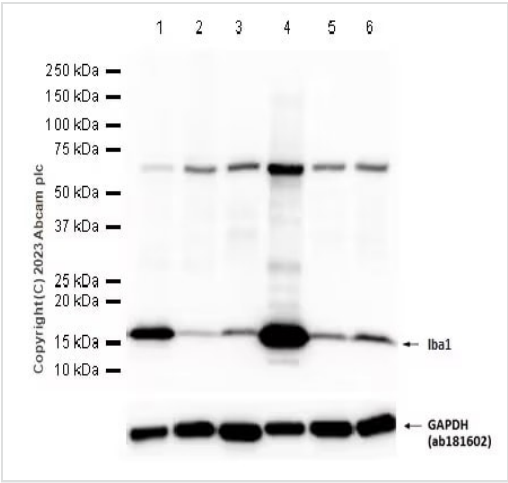
Flow Cytometry (Intracellular) - Anti-Iba1 antibody [EPR16588] (ab178846)

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

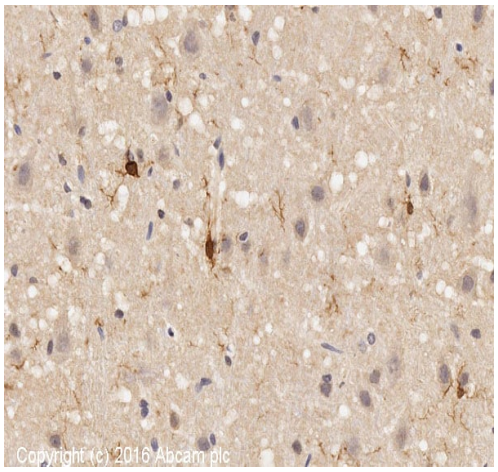
Exposure time: 40 seconds

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.

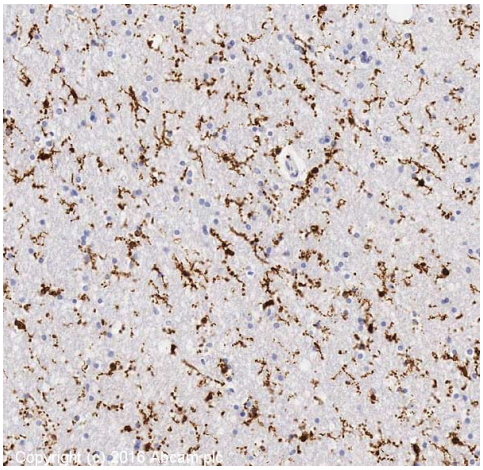
ab181602 was used as loading control.



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.

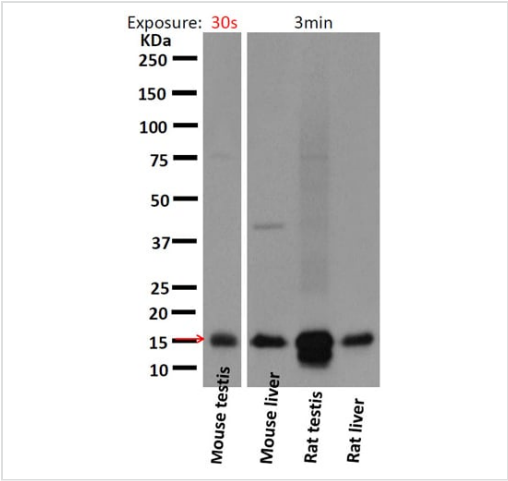
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



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)

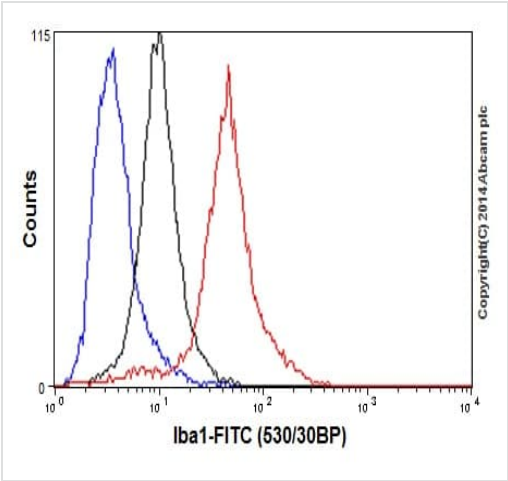
Lane 1 : Mouse testis

Lane 2 : Mouse liver

Lane 3 : Rat testis

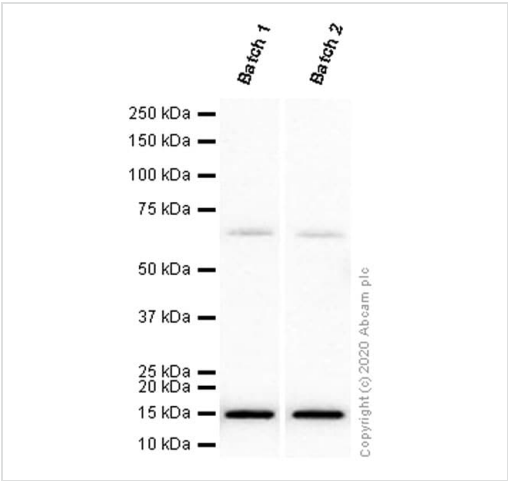
Lane 4 : Rat liver

Predicted band size: 17 kDa



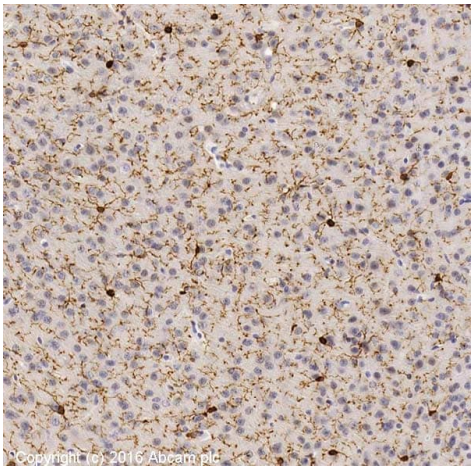
Flow Cytometry (Intracellular) - Anti-Iba1 antibody [EPR16588] (ab178846)

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)

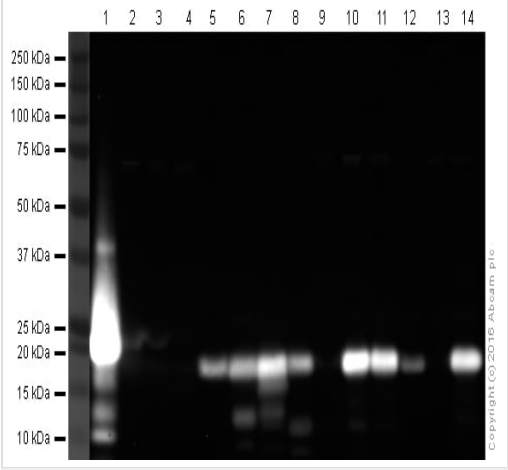
Different batches of ab178846 were tested on THP-1 (Human monocytic leukemia monocyte) lysate at 0.02 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 15 kDa.



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.

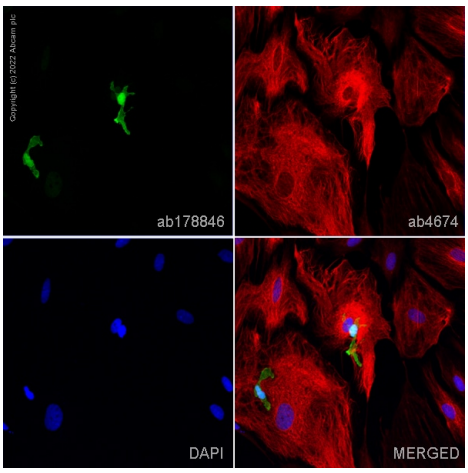
Performed under reducing conditions.

Predicted band size: 17 kDa

Exposure time: 1 minute

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

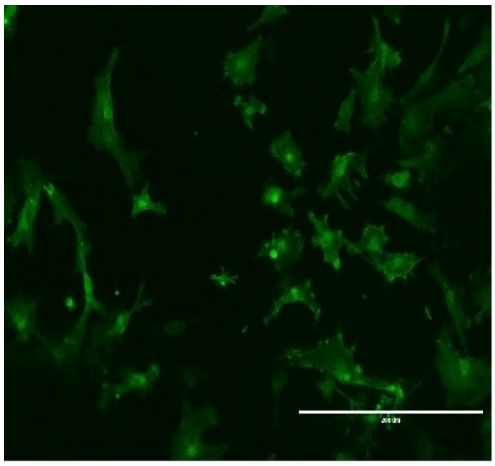
This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with ab178846 (anti-Iba1 antibody; 1/500 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)

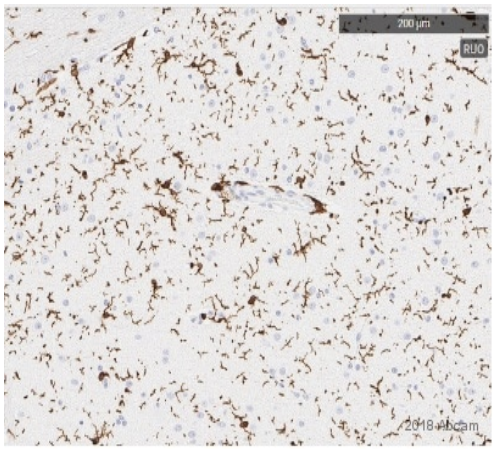
Immunofluorescence staining of Iba-1 using ab178846 in primary rat hippocampal mixed glia, (prepared from P2 rat hippocampal brain area, obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. SDPHP4m), DIV4. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton-X-100 (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).



Immunocytochemistry/ Immunofluorescence - Anti-Iba1 antibody [EPR16588] (ab178846)

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.

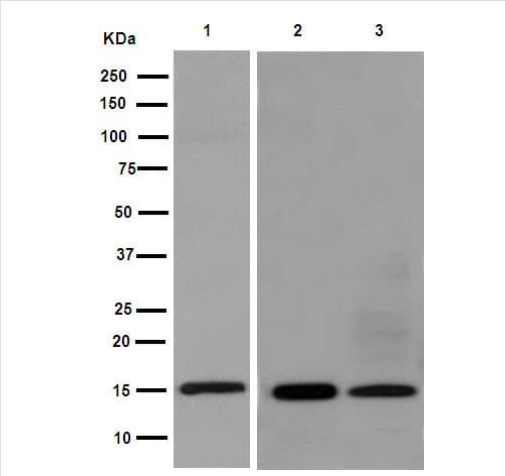


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16588] (ab178846)

This image was courtesy of an annonymous Abreview

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



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.

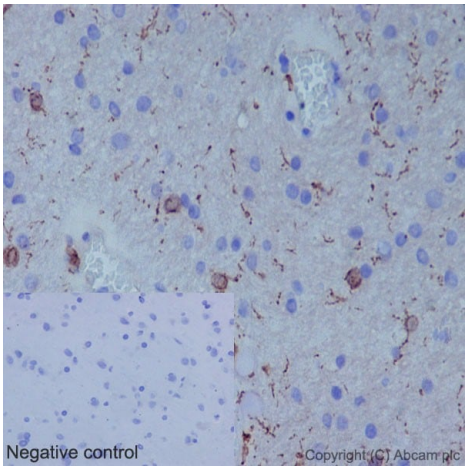
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Developed using the ECL technique.

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

Blocking buffer and concentration: 5% NFDM/TBST

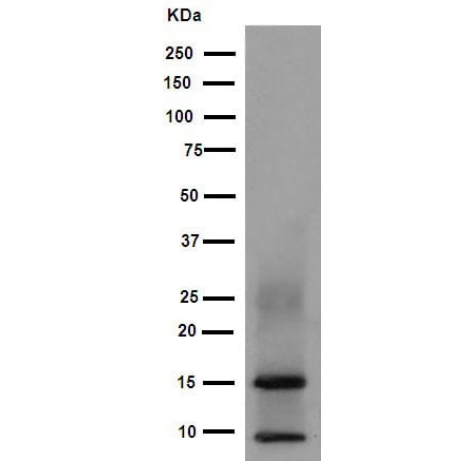
Diluting buffer and concentration: 5% NFDM /TBST



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

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.



Western blot - 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

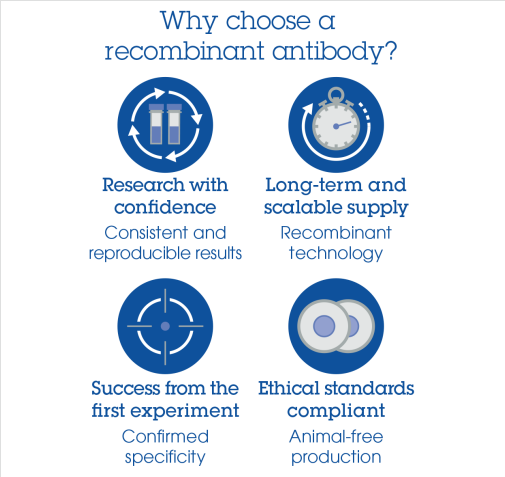
Predicted band size: 17 kDa
Observed band size: 10, 15 kDa

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.



Anti-Iba1 antibody [EPR16588] (ab178846)

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