# abcam

## Product datasheet

# Anti-Iba1 antibody [EPR16589] ab178847

Recombinant  RabMAb®

★★★★☆ **19 Abreviews**   **222 References**   14 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-Iba1 antibody [EPR16589] |
| **Description** | Rabbit monoclonal [EPR16589] to Iba1 |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** IHC (PFA fixed), IHC-P, WB, IP, ICC/IF |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human |
| **Immunogen** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | WB: Human spleen lysate; THP-1, MOLT-4 and U937 whole cell lysates; Mouse and rat spleen and testis lysates; Mouse hippocampus and brain lysates. IHC-P: Human cerebrum, mouse endometrium and rat cerebrum tissues. ICC/IF: U937 and THP-1 cells. IP: Mouse spleen whole cell lysate. IHC-Fr: Mouse hippocampus tissue. |
| **General notes** | This product is a recombinant monoclonal antibody, which offers several advantages including: <br><br>- High batch-to-batch consistency and reproducibility <br>- Improved sensitivity and specificity <br>- Long-term security of supply <br>- Animal-free production <br>For more information **see here**. <br><br>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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. |
| **Storage buffer** | Preservative: 0.01% Sodium azide <br> Constituents: 0.05% BSA, 40% Glycerol, 59% PBS |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR16589 |

| Isotype | IgG |
|---|---|

## Applications

Our **Abpromise guarantee** covers the use of ab178847 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 |
|---|---|---|
| **IHC (PFA fixed)** | | Use at an assay dependent concentration. |
| **IHC-P** | ★★★★★ (8) | 1/8000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **WB** | ★★★★☆ (3) | 1/1000. Detects a band of approximately 17 kDa (predicted molecular weight: 17 kDa). |
| **IP** | | 1/40. |
| **ICC/IF** | ★★★★☆ (3) | 1/100. |

## 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
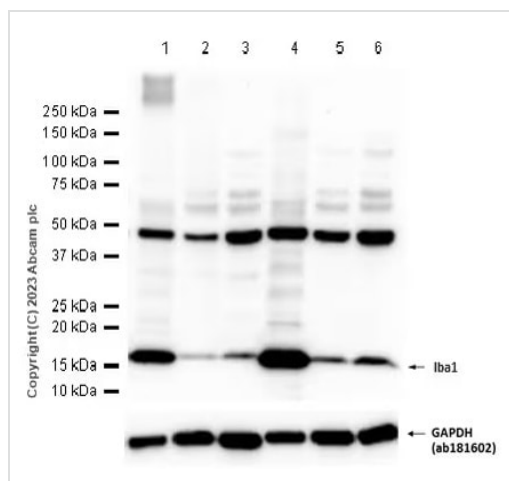
Western blot - Anti-Iba1 antibody [EPR16589] (ab178847)

**All lanes :** Anti-Iba1 antibody [EPR16589] (ab178847) at 1/1000 dilution

**Lane 1 :** Mouse spleen tissue lysate

**Lane 2 :** Mouse brain tissue lysate

**Lane 3 :** Mouse hippocampus tissue

**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
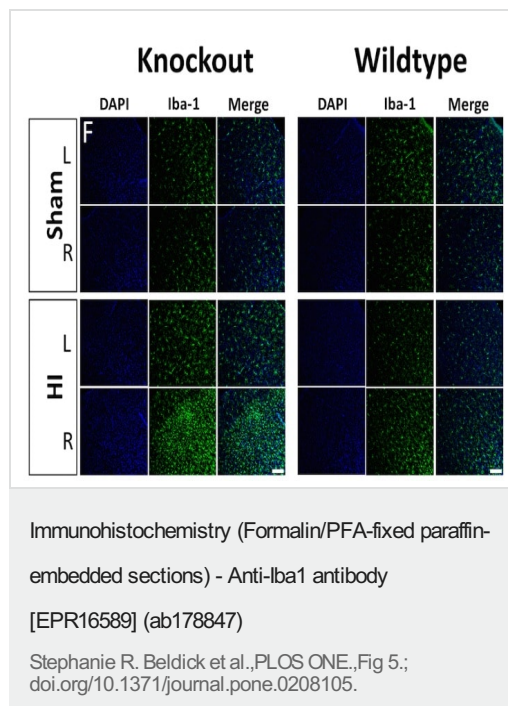
**Predicted band size:** 17 kDa

**Observed band size:** 17 kDa

**Exposure time:** 40 seconds

Blocking/Dilution buffer: 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.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16589] (ab178847)

Slides were washed with 1x PBS and then blocked in 5% goat serum containing 0.3% Triton X-100. Iba-1 was stained using ab178847 at 1/500 dilution in immunohistochemical analysis. Representative immunofluorescence is depicted for KO Sham (F top left), KO injured (F bottom left), WT Sham (F top right), and WT injured (F bottom right) in the right and left hemispheres (blue = DAPI, green = Iba-1). Scale bars = 100µm, 20x. HI = hypoxic-ischemic brain injury.

Area occupied by Iba-1-positive staining was elevated in KO injured animals when compared to uninjured controls, while stain area was not elevated in WT injured animals



Immunocytochemistry/ Immunofluorescence - Anti-Iba1 antibody [EPR16589] (ab178847)
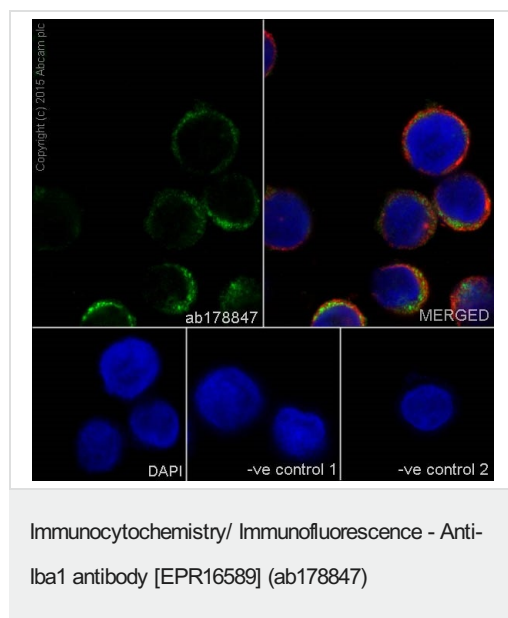
Immunofluorescent analysis of 100% methanol-fixed, 0.1% Triton X-100 permeabilized U937 (Human histiocytic lymphoma cell line) cells labeling Iba1 with ab178847 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on U937 cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG (AlexaFluor®594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab178847 at 1/100 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.

Immunoprecipitation - Anti-Iba1 antibody
[EPR16589] (ab178847)
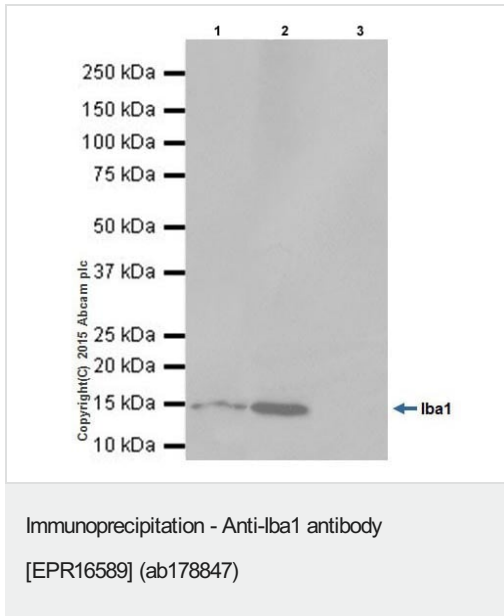
Iba1 was immunoprecipitated from 1mg of Mouse spleen whole cell lysate with ab178847 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab178847 at 1/1000 dilution.

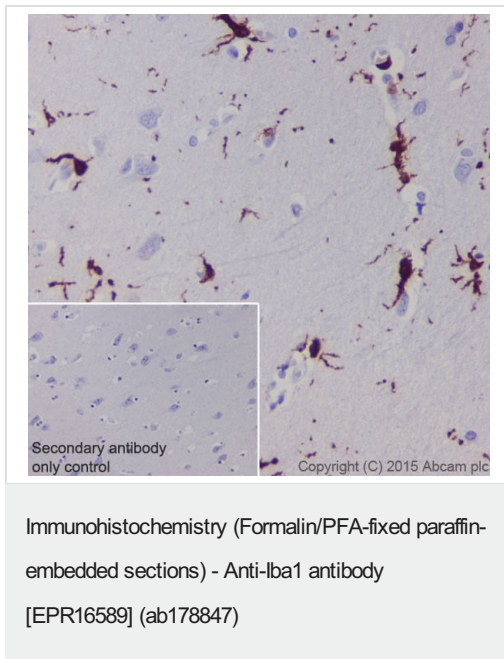VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: Mouse spleen whole cell lysate 10µg (Input).

Lane 2: ab178847 IP in Mouse spleen whole cell lysate.

Lane 3: Rabbit IgG,monoclonal-Isotype Control (**ab172730**) instead of ab178847 in Mouse spleen whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 5 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody
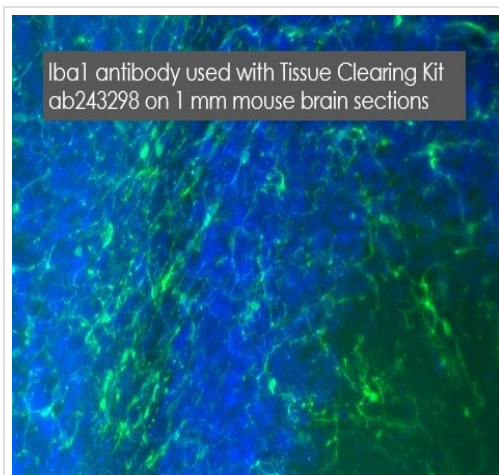[EPR16589] (ab178847)

Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling Iba1 with ab178847 at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Cytoplasm staining on microglia of the normal Human cerebrum is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Iba1 antibody ab178847 was used with Tissue Clearing Kit **ab243298** to penetrate, stain and clear a 1 mm coronal section of mouse brain. Blue: DAPI , Green: Iba1.

Learn more about **tissue clearing kits, reagents, and protocols** designed to make it easier to stain thick tissue sections and get more data from each valuable tissue section.

For 1 mm brain sections, we recommend a starting dilution of 1:100, and also using Goat Anti-Rabbit IgG H&L AlexaFluor488 (**ab150077**) at a dilution of 1:400.
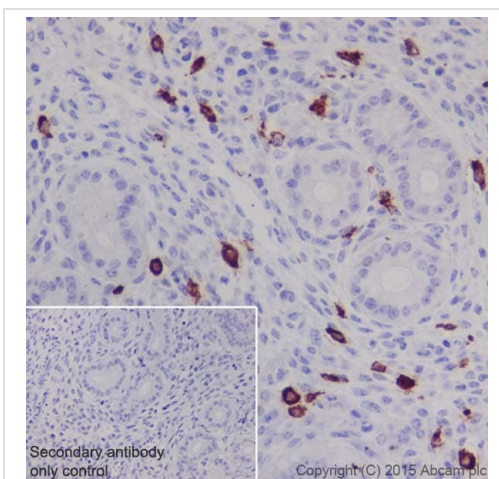
Immunohistochemistry (PFA fixed) - Anti-Iba1 antibody [EPR16589] (ab178847)



Immunohistochemical analysis of paraffin-embedded Mouse endometrium tissue labeling Iba1 with ab178847 at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Cytoplasm staining on macrophages of the mouse endometrium.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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 [EPR16589] (ab178847)
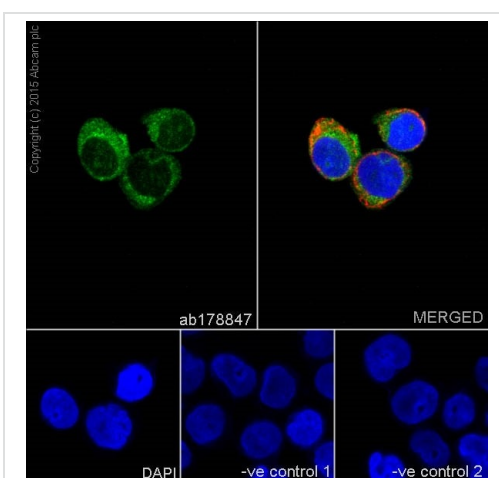


Immunofluorescent analysis of 100% methanol-fixed, 0.1% Triton X-100 permeabilized THP-1 (Human monocytic leukemia cell line) cells labeling Iba1 with ab178847 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on THP-1 cell line.

The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab178847 at 1/100 dilution followed by **ab150120** at

Immunocytochemistry/ Immunofluorescence - Anti-Iba1 antibody [EPR16589] (ab178847)

1/1000 dilution.

-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.
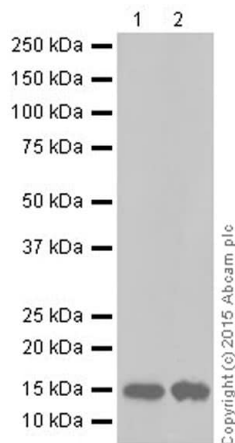


Western blot - Anti-Iba1 antibody [EPR16589] (ab178847)

**All lanes :** Anti-Iba1 antibody [EPR16589] (ab178847) at 1/2000 dilution

**Lane 1 :** Human spleen lysate

**Lane 2 :** THP-1 (Human monocytic leukemia cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes :** Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution
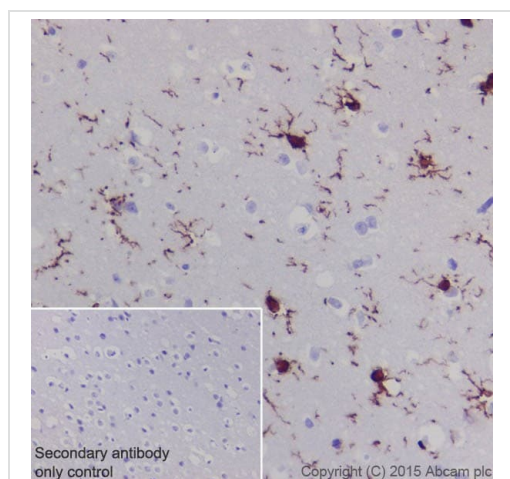
**Predicted band size:** 17 kDa

**Observed band size:** 17 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Different batches of ab178847 were tested on U-937 (Human histiocytic lymphoma monocyte) lysate at 0.5 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 17 kDa.



Western blot - Anti-Iba1 antibody [EPR16589] (ab178847)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody [EPR16589] (ab178847)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling Iba1 with ab178847 at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Cytoplasm staining on microglia of the rat cerebrum is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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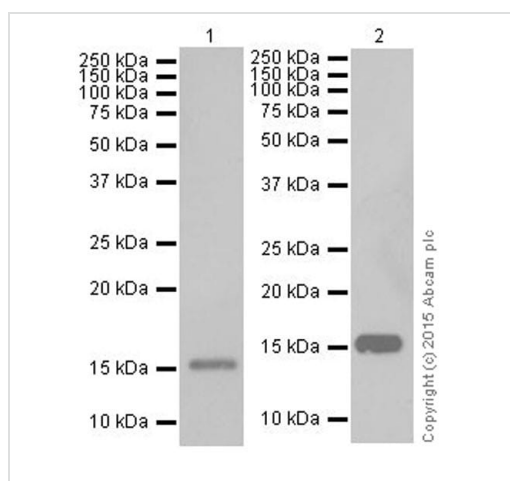


Western blot - Anti-Iba1 antibody [EPR16589] (ab178847)

**All lanes :** Anti-Iba1 antibody [EPR16589] (ab178847) at 1/1000 dilution

**Lane 1 :** MOLT-4 (Human lymphoblastic leukemia cell line) whole cell lysate
**Lane 2 :** U937 (Human histiocytic lymphoma cell line) whole cell lysate

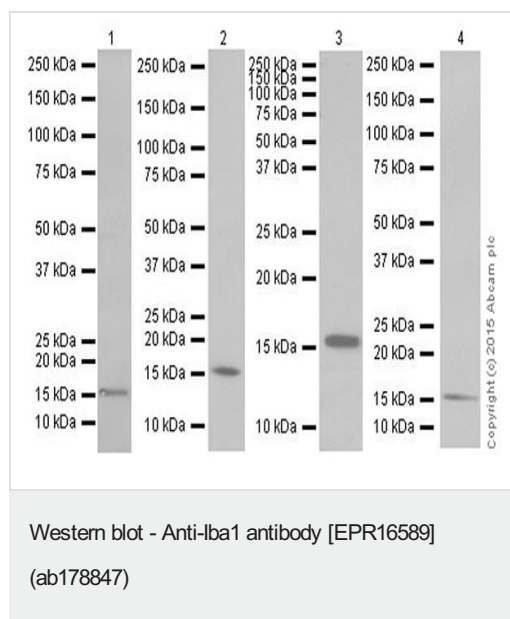Lysates/proteins at 10 µg per lane.

**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

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

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Western blot - Anti-Iba1 antibody [EPR16589]
(ab178847)

**All lanes :** Anti-Iba1 antibody [EPR16589] (ab178847) at 1/1000 dilution

**Lane 1 :** Mouse spleen lysate

**Lane 2 :** Rat spleen lysate

**Lane 3 :** Mouse testis lysate

**Lane 4 :** Rat testis lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

**Predicted band size:** 17 kDa

**Observed band size:** 17 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 1 minute; Lane 3 and 4: 3 minutes.



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 [EPR16589] (ab178847)

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