abcam

Product datasheet

Anti-Iba1 antibody [EPR16589] ab178847

יעלאעבע RabMAb

★★★★★ 19 Abreviews 222 References 画像数 14

製品の概要

製品名 Anti-lba1 antibody [EPR16589]

製品の詳細 Rabbit monoclonal [EPR16589] to lba1

由来種 Rabbit

アプリケーション 適用あり: IHC (PFA fixed), IHC-P, WB, IP, 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: 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.

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

精製度 Protein A purified

ポリモノ モノクローナル クローン名 EPR16589

アプリケーション

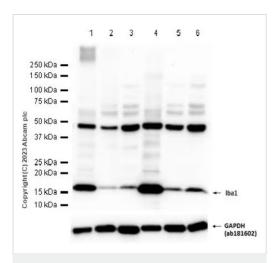
The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab178847の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション | Abreviews | 特記事項 |
|-----------------|-----------------|--|
| 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. |

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



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

All lanes : Anti-lba1 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 lgG H&L (HRP) (ab97051) at 1/20000

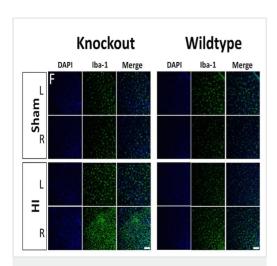
dilution

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.

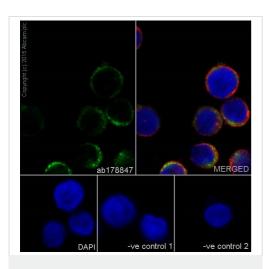


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Iba1 antibody
[EPR16589] (ab178847)

Stephanie R. Beldick et al.,PLOS ONE.,Fig 5.; doi.org/10.1371/journal.pone.0208105.

Slides were washed with 1x PBS and then blocked in 5% goat serum containing 0.3% Triton X-100. lba-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 = lba-1). Scale bars = $100\mu m$, 20x. HI = hypoxic-ischemic brain injury.

Area occupied by lba-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 lba1 with ab178847 at 1/100 dilution, followed by Goat Anti-Rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on U937 cell line.

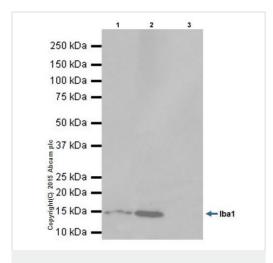
The nuclear counterstain is DAPI (blue).

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).

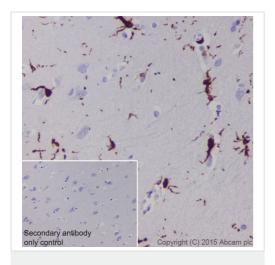
The negative controls are as follows:-

-ve control 1: ab178847 at 1/100 dilution followed by $\underline{ab150120}$ at 1/1000 dilution.

-ve control 2: <u>ab7291</u> at 1/1000 dilution followed by <u>ab150077</u> at 1/1000 dilution.



Immunoprecipitation - Anti-Iba1 antibody [EPR16589] (ab178847)



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

lba1 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) (<u>ab131366</u>), 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 lgG,monoclonal-lsotype Control (<u>ab172730</u>) instead of ab178847 in Mouse spleen whole cell lysate.

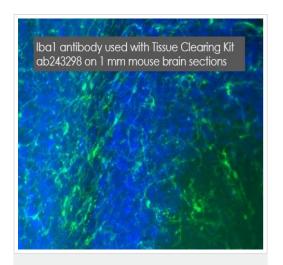
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 5 seconds.

Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling lba1 with ab178847 at 1/8000 dilution, followed by Goat Anti-Rabbit lgG 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.

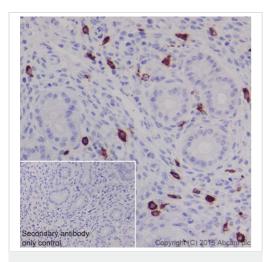


Immunohistochemistry (PFA fixed) - Anti-lba1 antibody [EPR16589] (ab178847)

lba1 antibody ab178847 was used with Tissue Clearing
Kit <u>ab243298</u> to penetrate, stain and clear a 1 mm coronal section
of mouse brain. Blue: DAPI, Green: lba1.

Learn more about <u>tissue clearing kits, reagents, and</u>
<u>protocols</u> 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 lgG H&L AlexaFluor488 (ab150077) at a dilution of 1:400.



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

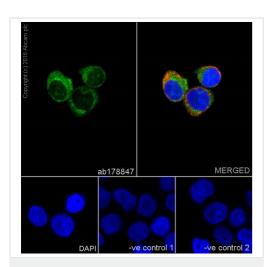
Immunohistochemical analysis of paraffin-embedded Mouse endometrium tissue labeling lba1 with ab178847 at 1/8000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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 lgG 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.



Immunocytochemistry/ Immunofluorescence - 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 lba1 with ab178847 at 1/100 dilution, followed by Goat anti-rabbit lgG (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 $\underline{ab150120}$ at 1/1000 dilution.

-ve control 2: $\underline{ab7291}$ at 1/1000 dilution followed by $\underline{ab150077}$ at 1/1000 dilution.

1 2
250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

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

All lanes : Anti-lba1 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

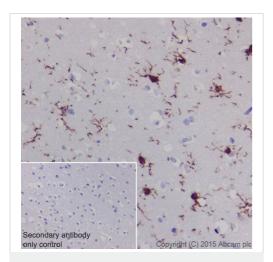
Predicted band size: 17 kDa **Observed band size:** 17 kDa

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 μ g/ml. 15 μ g of lysate was loaded in each lane. Bands observed at 17 kDa.



(ab178847)

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

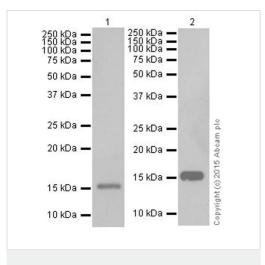
Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling lba1 with ab178847 at 1/8000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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 lgG 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-lba1 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) (<u>ab97051</u>) at 1/10000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

250 kDa -250 kDa -250 kDa -150 kDa -150 kDa -150 kDa -100 kDa -100 kDa -50 kDa -100 kDa -37 kDa -75 kDa -75 kDa -75 kDa -50 kDa -50 kDa 🕳 50 kDa -25 kDa -37 kDa -37 kDa -37 kDa -20 kDa -25 kDa -25 kDa -25 kDa — 20 kDa — 20 kDa -15 kDa -20 kDa -15 kDa 🕳 15 kDa • 15 kDa -10 kDa 10 kDa -10 kDa = 10 kDa 🕳

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

All lanes : Anti-lba1 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) (<u>ab97051</u>) 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.



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