abcam

Product datasheet

Anti-Iba1 antibody [EPR16589] ab178847

重组 RabMAb

★★★★★ 19 Abreviews 222 References 14 图像

概述	
产品名称	Anti-lba1 抗体 [EPR16589]
描述	免 单 克隆抗体 [EPR16589] to lba1
宿主	Rabbit
经测试应 用	适用于: IHC (PFA fixed), IHC-P, WB, IP, 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: 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.
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 <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>.

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: 0.05% BSA, 40% Glycerol, 59% PBS
纯 度	Protein A purified
克隆	单 克隆
克隆 编号	EPR16589

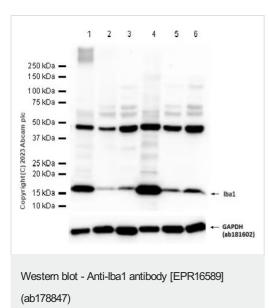
应用

The Abpromise guarantee Abpromise ™承诺保证使用ab178847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
IHC (PFA fixed)		Use at an assay dependent concentration.
IHC-P	★ ★ ★ ★ ★ <u>(8)</u>	1/8000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★ ★ ★ ★ ☆ (<u>3)</u>	1/1000. Detects a band of approximately 17 kDa (predicted molecular weight: 17 kDa).
IP		1/40.
ICC/IF	★★★★★ (<u>3)</u>	1/100.

靶 标					
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.				



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

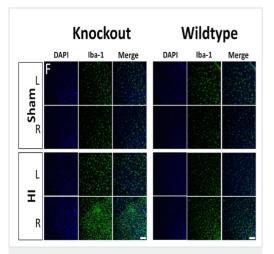
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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.

ab178847

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

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 = hypoxicischemic 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

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 IgG (Alexa Fluor® 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on U937 cell line.

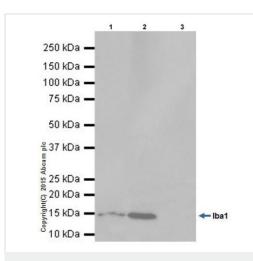
The nuclear counterstain is DAPI (blue).

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

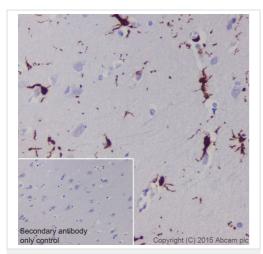
The negative controls are as follows:-

-ve control 1: ab178847 at 1/100 dilution followed by <u>ab150120</u> at 1/1000 dilution.

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







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 (**ab172730**) instead of ab178847 in Mouse spleen whole cell lysate.

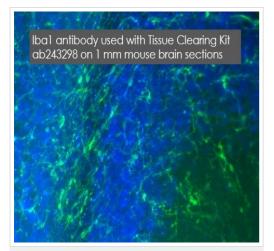
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

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 IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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

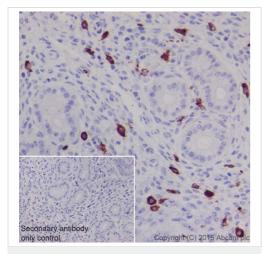
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution.



Immunohistochemistry (PFA fixed) - Anti-Iba1 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> 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 lgG H&L AlexaFluor488 (**ab150077**) at a dilution of 1:400.



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

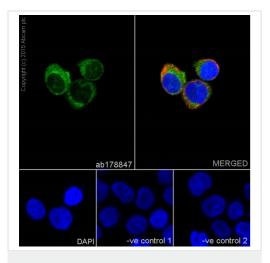
Immunohistochemical analysis of paraffin-embedded Mouse endometrium tissue labeling lba1 with ab178847 at 1/8000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Cytoplasm staining on macrophages of the mouse endometrium.

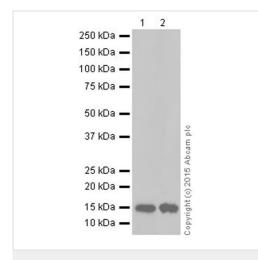
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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)



Western blot - 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 IgG (Alexa Fluor® 488) (<u>ab150077</u>) 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 <u>ab150120</u> at 1/1000 dilution.

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

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

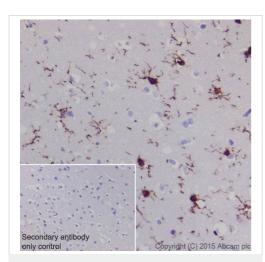
Exposure time: 3 minutes

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.

Western blot - Anti-Iba1 antibody [EPR16589] (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 IgG H&L (HRP) (<u>ab97051</u>) 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) (<u>ab97051</u>) 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 lgG 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.

	1		2		3		4
50 kDa 🗕		250 kDa 🗕		258 kBa ≡		250 kDa 🗕	
50 kDa 🗕		150 kDa 🗕		100 kDa — 75 kDa —		150 kDa 🗕	
00 kDa 🗕		100 kDa 🗕		50 kDa 🗕		100 kDa 🗕	
75 kDa 🗕		75 kDa 🗕		37 kDa 🗕		75 kDa 🗕	
50 kDa 🗕		50 kDa 🗕		25 kDa 🗕		50 kDa 🗕	
37 kDa 🗕		37 kDa 🗕		20 kDa 🗕		37 kDa 🗕	
		25 kDa 🗕				25 kDa 🗕	
25 kDa 🗕		20 kDa 🗕		15 kDa 🗕	-	20 kDa -	
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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 lgG 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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