abcam

Product datasheet

Anti-Iba1 antibody ab153696

2 Abreviews 27 References 12 Images

Overview

Product name	Anti-Iba1 antibody
Description	Rabbit polyclonal to Iba1
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-P, IP, WB, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment corresponding to Human Iba1 (internal sequence). Database link: **P55008**
Positive control	WB: Rat and mouse liver extract, THP-1, mouse and rat brain tissue; IHC-P: Rat and mouse brain tissue; Flow: human THP1 cells, murine microglia cells; ICC/IF: human THP-1 cells; IP: human K562 cells
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.00 Preservative: 0.025% Proclin 300 Constituents: 79% PBS, 20% Glycerol (glycerin, glycerine)
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab153696 in the following tested applications.

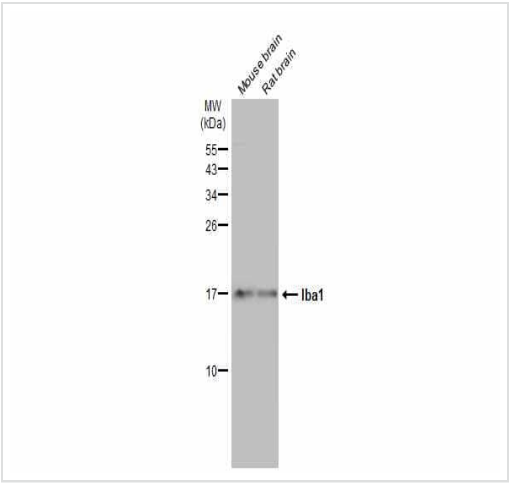
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/100 - 1/1000. **Not suitable for mouse**
IHC-P		1/100 - 1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/100 - 1/500.
WB		1/500 - 1/10000. Predicted molecular weight: 16 kDa. For ab153696 Abcam recommends blocking in 5% milk for cleanest results in WB. Blocking with BSA gives slightly higher background.
Flow Cyt		1/50 - 1/200. **ab171870** - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.

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 (ab153696)

All lanes : Anti-Iba1 antibody (ab153696) at 1/1000 dilution

Lane 1 : Mouse brain

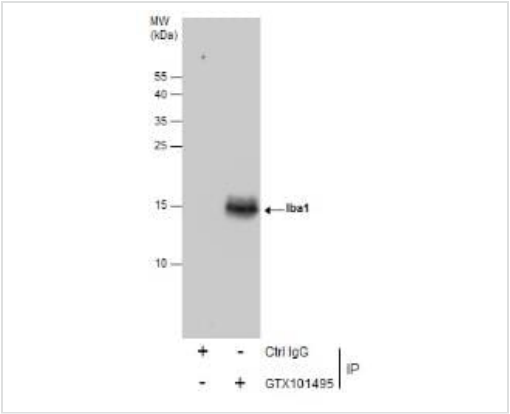
Lane 2 : Rat brain

Lysates/proteins at 50 µg/ml per lane.

Secondary

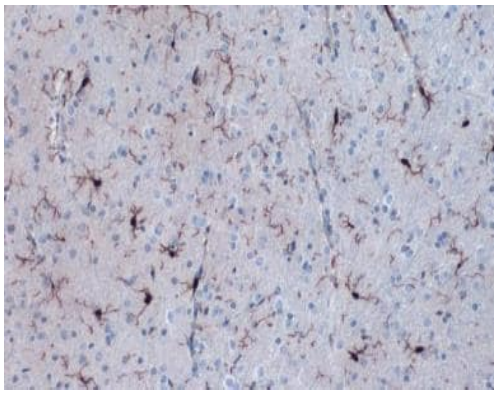
All lanes : HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 16 kDa



Immunoprecipitation - Anti-Iba1 antibody (ab153696)

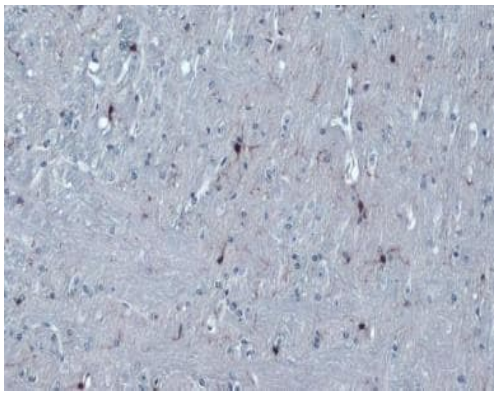
Immunoprecipitation of K562 whole cell extract (human chronic myelogenous leukemia cell line from bone marrow) using 5µg/ml ab153696 to detect Iba1 protein with secondary: anti-Rabbit IgG.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody (ab153696)

Immunohistochemistry of paraffin-embedded rat cerebellum tissue staining Iba1 protein with ab153696 at 1/1000 dilution.

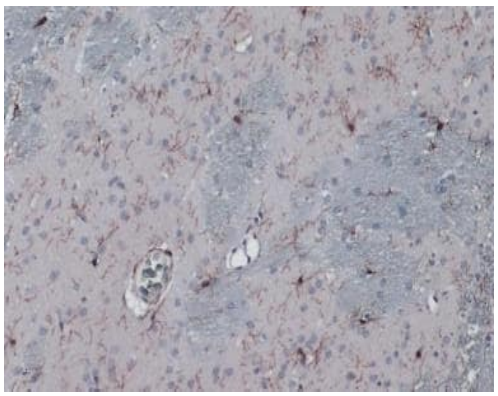
Performed with heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody (ab153696)

Immunohistochemistry of paraffin-embedded mouse cerebellum tissue staining Iba1 protein with ab153696 at 1/1000 dilution.

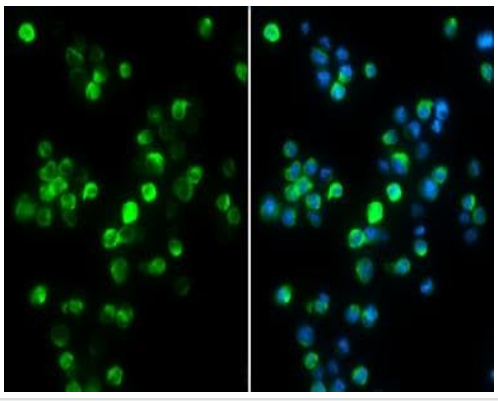
Performed with heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody (ab153696)

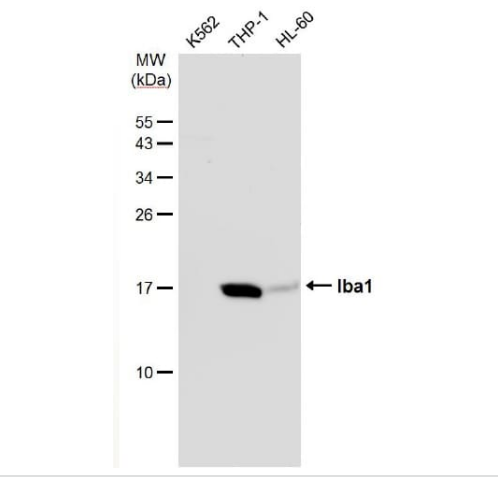
Immunohistochemistry of paraffin-embedded rat brain tissue staining Iba1 protein with ab153696 at 1/2000 dilution.

Performed with heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Iba1 antibody (ab153696)

Immunocytochemical analysis of THP-1 cells (human monocytic leukemia cell line) staining cytoplasmic Iba1 protein with ab153696 in 1/1000 dilution (green). Counterstaining (blue) using fluoroshield with DAPI.



Western blot - Anti-Iba1 antibody (ab153696)

All lanes : Anti-Iba1 antibody (ab153696) at 1/500 dilution

Lane 1 : K562 whole cell extracts
Lane 2 : THP-1 whole cell extracts
Lane 3 : HL-60 whole cell extracts

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : HRP-conjugated anti-rabbit IgG antibody at 1/10000 dilution

Developed using the ECL technique.

Predicted band size: 16 kDa

High expression in THP-1. Low expression in K562 and HL-60.

15% SDS-PAGE.

Running condition: 80V, 15min; 140V, 40 min.

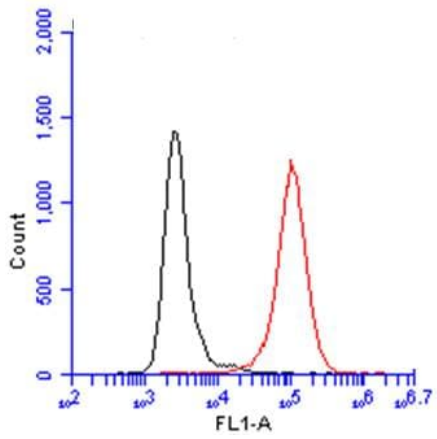
Transfer condition: Semi-dry, 18 V, 60 min (Nitrocellulose membrane).

Blocking condition: 5% non-fat milk in TBST, RT, 60 min.

Primary antibody incubation: 4°C overnight.

Washing condition: 5 ml TBST, 4 x 5 min.

ECL exposure.

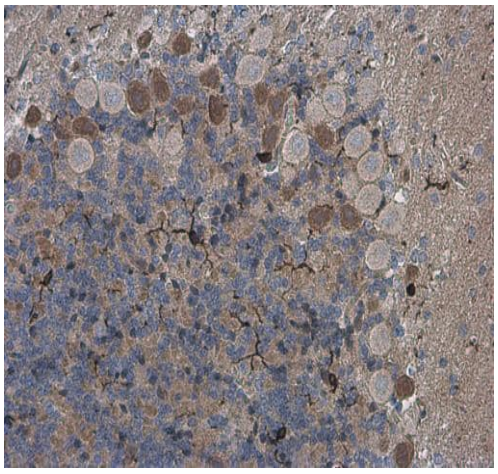


Flow Cytometry - Anti-Iba1 antibody (ab153696)

Flow cytometric analysis of THP-1 (human monocytic leukemia cell line) cell line labeling Iba1 with ab153696 at 1/50 dilution (red) compared with an unlabelled sample (black).

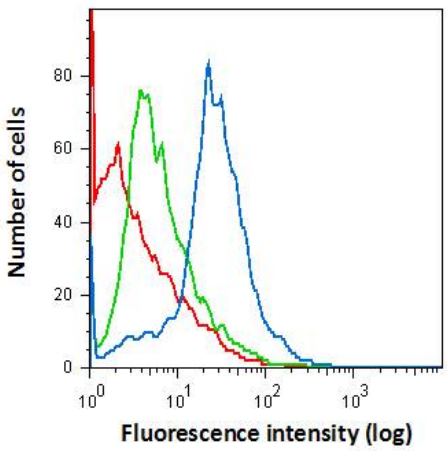
The sample was fixed using 4% PFA in PBS at 4▌ for 15 minutes. Cells were resuspended twice in 0.1% Triton X-100 in PBS (wash buffer), then centrifuged at 4▌ for 5 mintues. The sample was incubated with the primary antibody (1/50 in PBS) for 60 minutes at 4°C. A. Alexa Fluor® 488-conjugated anti-rabbit IgG (1/1000 in PBS) at 4°C for 30 minutes was used as the secondary antibody.

Analysed using a BD Accuri™ C6 Cytometer.



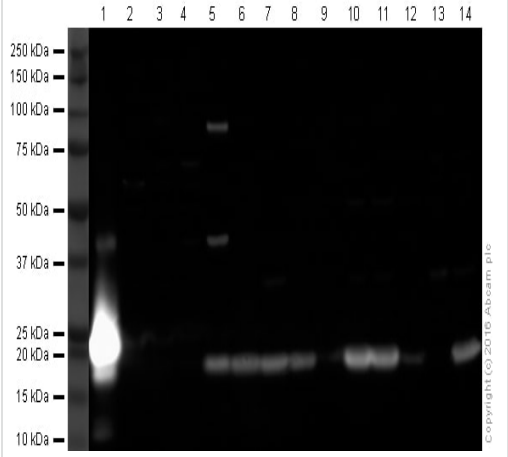
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody (ab153696)

Paraffin-embedded rat brain tissue stained for Iba1 using ab153696 at 1/500 dilution in immunohistochemical analysis. Antigen retrieval: Citrate buffer, pH 6.0, 15 min



Flow Cytometry - Anti-Iba1 antibody (ab153696)

Flow cytometic analysis of primary murine microglia cells labeling Iba1 with ab153696 at 1.0 µg per 4×10^5 cells (blue), Rabbit IgG (green) , Unstained (red).



Western blot - Anti-Iba1 antibody (ab153696)

All lanes : Anti-Iba1 antibody (ab153696) at 1/500 dilution

Lane 1 : Human Iba1 full length recombinant protein at 0.1 µg
Lane 2 : HEK293 whole cell lysate at 20 µg
Lane 3 : A431 whole cell lysate at 20 µg
Lane 4 : NIH3T3 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 whole cell lysate at 30 µg
Lane 9 : MOLT4 whole cell lysate at 30 µg
Lane 10 : THP1 whole cell lysate at 30 µg
Lane 11 : THP1 whole cell lysate, PMA treated at 30 µg
Lane 12 : Raw 264.7 whole cell lysate at 30 µg
Lane 13 : C6 whole cell lysate at 30 µg
Lane 14 : NR8383 whole cell lysate at 30 µg

Developed using the ECL technique.

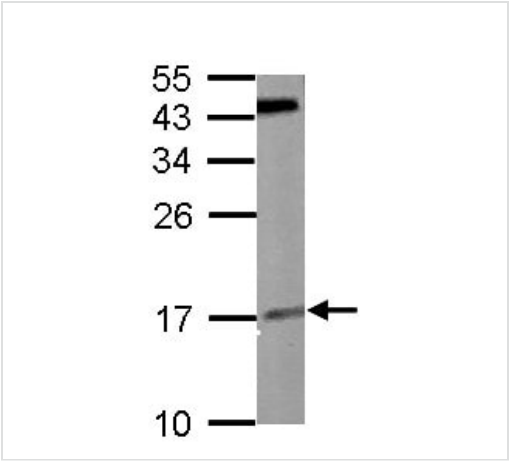
Performed under reducing conditions.

Predicted band size: 16 kDa

Exposure time: 1 minute

BLOCKED IN 3% MILK. For ab153696 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 ab153696 (anti-Iba1 antibody; 1/500 dilution) for 18 hours at 4°C. Antibody binding was detected using HRP-labelled anti-Rabbit IgG for 1 hour at room temperature and visualised using ECL development solution **ab133406**.



Western blot - Anti-Iba1 antibody (ab153696)

Anti-Iba1 antibody (ab153696) at 1/1000 dilution + Mouse liver whole cell lysate at 50 µg

Secondary
Rabbit IgG antibody (HRP) for 1 hour at room temperature at 1/10000 dilution

Predicted band size: 16 kDa

15% SDS PAGE

Running conditions: 80V for 15min then 140V for 40min

Blocking: 5% non-fat milk in TBST at room temperature for 60min.

Washing conditions: 5 ml TBST, 4 x 5min

Transfer conditions: Semi-dry, 18 V, 60min (NC membrane)

Exposure system: Trident plus Western HRP Substrate

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