abcam

Product datasheet

Anti-CD11b antibody [EP1345Y] - C-terminal ab52478

Recombinant RabMAb

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Overview

Product name	Anti-CD11b antibody [EP1345Y] - C-terminal
Description	Rabbit monoclonal [EP1345Y] to CD11b - C-terminal
Host species	Rabbit
Specificity	Testing of mouse and rat tissues (brain, spleen, kidney and heart) in WB gave negative results. However, flow cytometry for mouse RAW 264.7 cell line gave positive results. We have not tested any rat samples in flow cytometry. Due to the variability in mouse, we do not list this as a tested species. We welcome any feedback on mouse and rat reactivity.
Tested applications	**Suitable for:** ICC/IF, IHC-FoFr, WB, IP, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human CD11b aa 1100 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **P11215**
Positive control	IHC-P: Human spleen and human cervical cancer tissue WB: TF1 lysate. THP-1 macrophages, +10 ng/ml LPS. ICC/IF: THP-1 cell lysate IP: TF-1 whole cell lysate
General notes	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**.

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	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1345Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52478 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 - 1/250.
IHC-FoFr	★★★★☆ (1)	Use at an assay dependent concentration.
WB	★★★★★ (2)	1/1000. Predicted molecular weight: 128 kDa. **For unpurified use at 1/20000 - 1/50000**
IP		1/30. **For unpurified use at 1/80**
IHC-P	★★★★★ (1)	1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified use at 1 - 5 µg/ml**

Target

Function Integrin alpha-M/beta-2 is implicated in various adhesive interactions of monocytes, macrophages and granulocytes as well as in mediating the uptake of complement-coated particles. It is identical with CR-3, the receptor for the iC3b fragment of the third complement component. It probably recognizes the R-G-D peptide in C3b. Integrin alpha-M/beta-2 is also a receptor for fibrinogen, factor X and ICAM1. It recognizes P1 and P2 peptides of fibrinogen gamma chain.

Tissue specificity Predominantly expressed in monocytes and granulocytes.

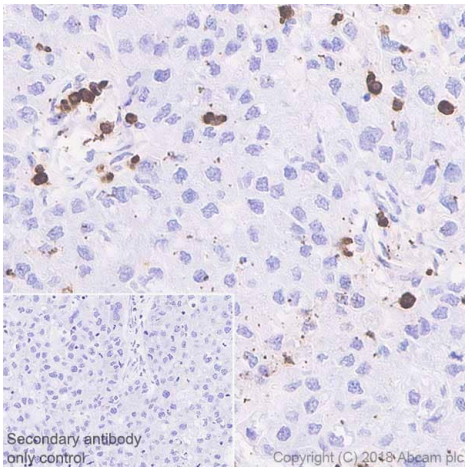
Involvement in disease Genetic variations in ITGAM has been associated with susceptibility to systemic lupus erythematosus type 6 (SLEB6) [MIM:609939]. Systemic lupus erythematosus (SLE) is a chronic, inflammatory and often febrile multisystemic disorder of connective tissue. It affects principally the skin, joints, kidneys and serosal membranes. It is thought to represent a failure of the regulatory mechanisms of the autoimmune system.

Sequence similarities Belongs to the integrin alpha chain family.
Contains 7 FG-GAP repeats.
Contains 1 VWFA domain.

Domain The integrin I-domain (insert) is a VWFA domain. Integrins with I-domains do not undergo protease cleavage.

Cellular localization Membrane.

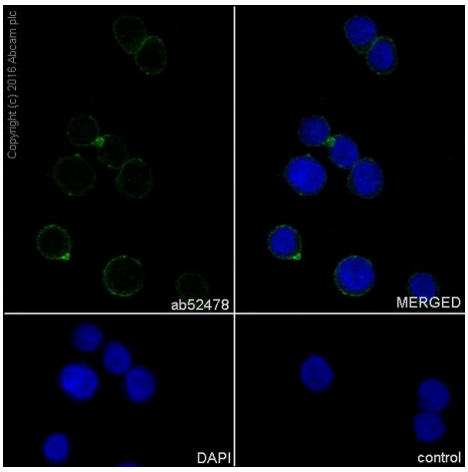
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical cancer tissue sections labeling CD11b with purified ab52478 at 1:1000 dilution (0.28 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0) ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Hematoxylin was used as a counterstain.

Negative control: PBS instead of the primary antibody (inset).



Immunocytochemistry/ Immunofluorescence - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

Unpurified ab52478 staining CD11b in the THP-1 (Human monocytic leukemia cell line) cell line by ICC/IF (Immunocytochemistry/immunofluorescence).

Cells were fixed with 100% methanol. Samples were incubated with primary antibody (1/250). **ab150077** was used as the secondary antibody (1/1000). Nuclei were stained with DAPI.

Western blot - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

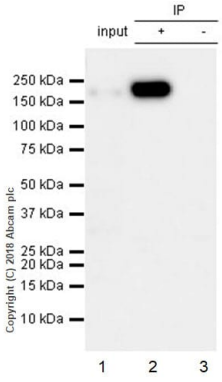
Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478) at 0.3 µg/ml (purified) + TF-1 (Human Erythroleukemia erythroblast) whole cell lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 128 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

ab52478 (purified) at 1:30 dilution (2 µg) immunoprecipitating CD11b in TF-1 (Human bone marrow erythroleukemia cell line) whole cell lysate.

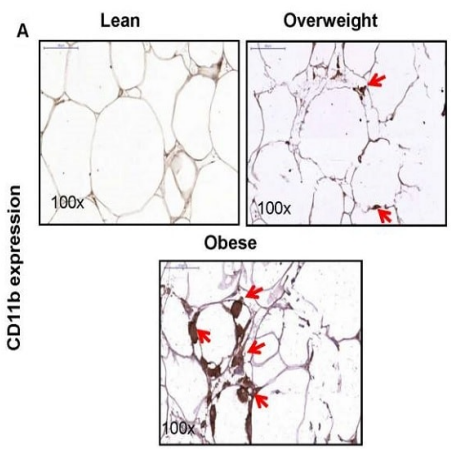
Lane 1: TF-1 whole cell lysate 10 µg (input).

Lane 2: ab52478 + TF-1 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab52478 in TF-1 whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



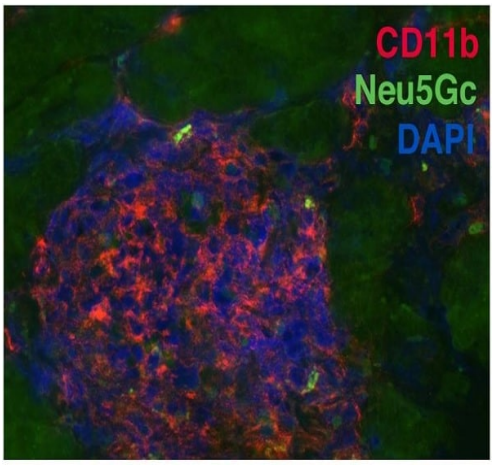
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

Sindhu et al PLoS One. 2015 Jul 22;10(7):e0133494. doi: 10.1371/journal.pone.0133494. eCollection 2015. Fig 5. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Enhanced expression of monocytes/ macrophage markers in the obese adipose tissue.

The protein expression (intensity) of monocyte/ macrophage markers was detected by immunohistochemistry (IHC) in the adipose tissue samples from lean, overweight, and obese individuals, 10 each. As shown by representative IHC photomicrographs (100× magnification), expression of (**A**) CD11b was found to be markedly elevated in overweight and obese adipose tissue samples as compared with lean samples.

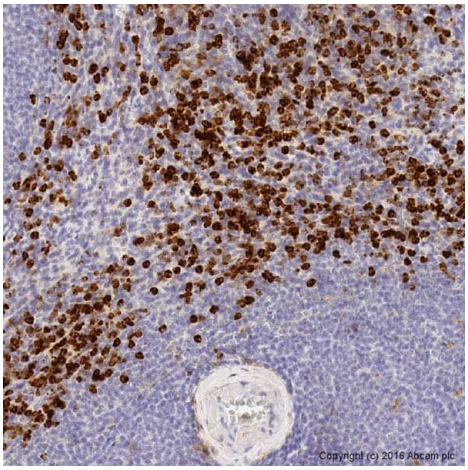
Paraffin-embedded sections (4 μm thick) of subcutaneous adipose tissue were deparaffinized in xylene and rehydrated through descending grades of ethanol (100%, 95%, and 75%) to water. Antigen retrieval was performed under pressure cooker boiling for 8 min and cooling for 15 min. After washing in PBS, endogenous peroxidase activity was blocked with 3% H_2O_2 for 30 min and non-specific antibody binding was clocked with 5% nonfat milk for 1hr and 1% bovine serum albumin (BSA) solution for 1hr. Slides were treated overnight with primary antibodies at room temperature. After washing with PBS (0.5% Tween), slides were incubated for 1hr with secondary antibody conjugated with HRP polymer chain and color was developed using 3,3'-diaminobenzidine chromogen substrate. Specimens were washed in running tap water, lightly counterstained with hematoxylin, dehydrated through ascending grades of ethanol (75%, 95%, and 100%), cleared in xylene, and finally mounted in dibutyl phthalate xylene (DPX).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

Duchenne muscular dystrophy (DMD) muscle was co-stained for Neu5Gc (green), ab52478 (red) and DAPI (blue).

For double immunostaining, sections were first stained overnight at 4°C with anti-Neu5Gc after blocking in 10% (Neu5Gc-free) human serum, after blocking in 5 mg/mL BSA, sections were incubated overnight with both primary antibodies without fixation, washed for one hour and incubated with the appropriate secondary antibodies.



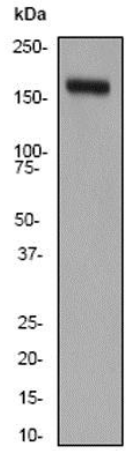
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

IHC image of CD11b staining in a formalin fixed, paraffin embedded normal human spleen 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 (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with unpurified ab52478, 5 µg/ml, for 15 minutes 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.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Western blot - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

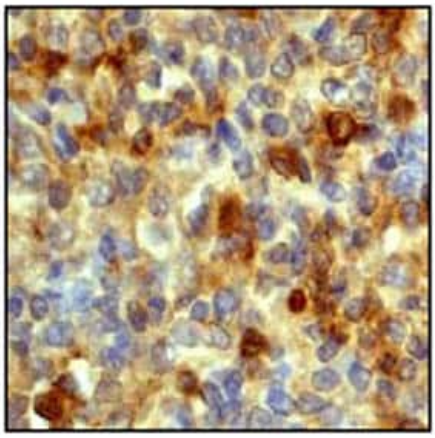
Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478) at 1/20000 dilution (unpurified) + TF-1 (Human bone marrow erythroleukemia cell line) lysate at 10 µg

Secondary

goat anti-rabbit HRP labeled at 1/2000 dilution

Predicted band size: 128 kDa

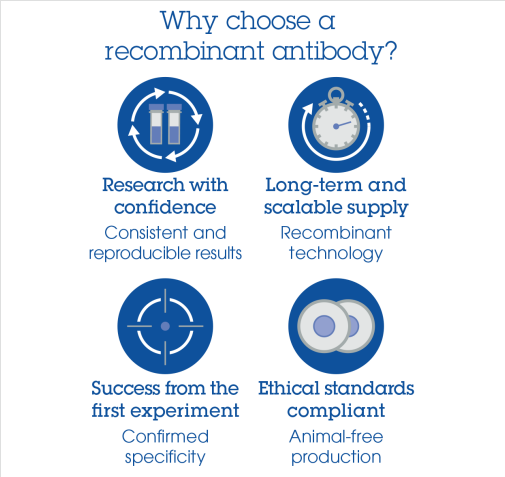
Observed band size: 170 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

Immunohistochemical analysis of paraffin-embedded human spleen tissue using unpurified ab52478 at a dilution of 1/100.

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



Anti-CD11b antibody [EP1345Y] - C-terminal (ab52478)

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