# abcam

# Product datasheet

# Anti-CD11b antibody [EP1345Y] - BSA and Azide free ab187537





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#### Overview

**Product name** Anti-CD11b antibody [EP1345Y] - BSA and Azide free

**Description** Rabbit monoclonal [EP1345Y] to CD11b - BSA and Azide free

**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. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Human spleen tissue WB: TF1 lysate

**General notes** ab187537 is the carrier-free version of ab52478.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEP1345Y

**Isotype** IgG

# **Applications**

# The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab187537 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		Use at an assay dependent concentration.
IHC-FoFr		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 128 kDa.
IP		Use at an assay dependent concentration.
IHC-P	<b>★★★★ (1)</b>	Use at an assay dependent concentration. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

#### **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.

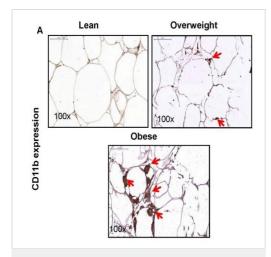
Contains 7 FG-GAP repeats. Contains 1 VWFA domain.

**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 paraffinembedded sections) - Anti-CD11b antibody

[EP1345Y] - BSA and Azide free (ab187537)

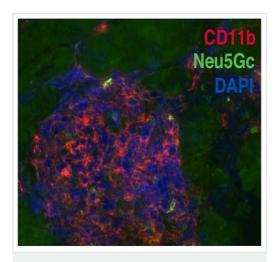
Image from Sindhu et al PLoS One. 2015 Jul 22;10(7):e0133494. doi: 10.1371/journal.pone.0133494. eCollection 2015. Fig 5.

# 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<sub>2</sub>O<sub>2</sub> for 30 min and nonspecific 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52478).



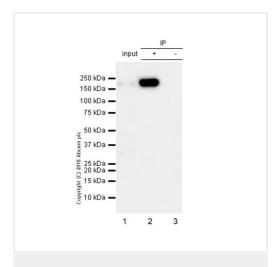
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD11b antibody
[EP1345Y] - BSA and Azide free (ab187537)

Image from Martin et al PLoS One. 2014 Feb 5;9(2):e88226. doi: 10.1371/journal.pone.0088226. eCollection 2014. Fig 6.

Duchenne muscular dystrophy (DMD) muscle was co-stained for Neu5Gc (green), <u>ab52478</u> (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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52478**).



Immunoprecipitation - Anti-CD11b antibody
[EP1345Y] - BSA and Azide free (ab187537)

<u>**ab52478**</u> (purified) at 1:30 dilution (2  $\mu$ g) 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

 $\textbf{Lane 3:} \ \ \text{Rabbit monoclonal lgG } (\underline{\textbf{ab172730}}) \ \text{instead of } \underline{\textbf{ab52478}} \ \text{in}$ 

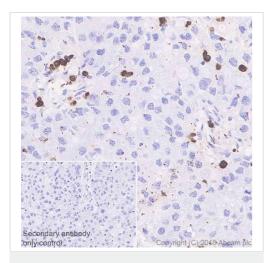
TF-1 whole cell lysate

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab52478</u>).



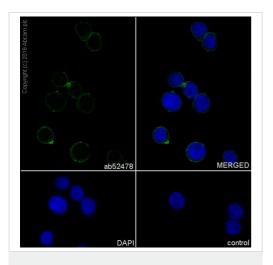
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD11b antibody

[EP1345Y] - BSA and Azide free (ab187537)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical cancer tissue sections labeling CD11b with purified <u>ab52478</u> at 1:1000 dilution (0.28 µg/ml). Heat mediated antigen retrieval was performed using <u>ab93684</u> (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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52478).

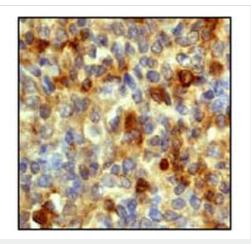


Immunocytochemistry/ Immunofluorescence - Anti-CD11b antibody [EP1345Y] - BSA and Azide free (ab187537)

Unpurified <u>ab52478</u> 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). <u>ab150077</u> was used as the secondary antibody (1/1000). Nuclei were stained with DAPI.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab52478</u>).

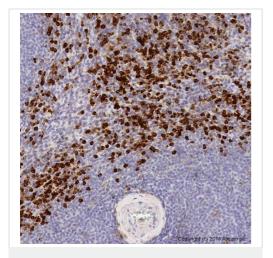


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD11b antibody

[EP1345Y] - BSA and Azide free (ab187537)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52478).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD11b antibody

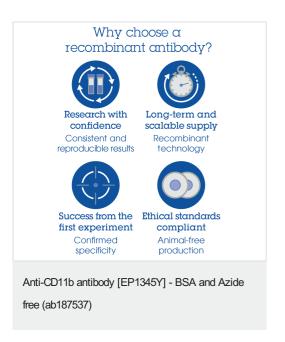
[EP1345Y] - BSA and Azide free (ab187537)

This IHC data was generated using the same anti-CD11b antibody clone, EP1345Y, in a different buffer formulation (cat# <u>ab52478</u>).

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 <u>ab52478</u>, 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



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