abcam

Product datasheet

Anti-CD86 antibody [EP1158-37] ab269587

KO VALIDATED RabMAb

5 References 5 Images

Overview

Product name Anti-CD86 antibody [EP1158-37]

Description Rabbit monoclonal [EP1158-37] to CD86

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Unsuitable for: Flow Cyt,ICC/IF or IP

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Raji, Daudi, Jurkat, Human tonsil and Human spleen lysates. IHC-P: Human non-Hodgkin

lymphoma and Human tonsil tissues.

General notes Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

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. 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: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EP1158-37

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab269587 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
WB		1/1000. Detects a band of approximately 80 kDa (predicted molecular weight: 38 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for Flow Cyt,ICC/IF or IP.

Target

Function Receptor involved in the costimulatory signal essential for T-lymphocyte proliferation and

interleukin-2 production, by binding CD28 or CTLA-4. May play a critical role in the early events of

T-cell activation and costimulation of naive T-cells, such as deciding between immunity and anergy that is made by T-cells within 24 hours after activation. Isoform 2 interferes with the formation of CD86 clusters, and thus acts as a negative regulator of T-cell activation.

Tissue specificity Expressed by activated B-lymphocytes and monocytes.

Sequence similarities Contains 1 lg-like C2-type (immunoglobulin-like) domain.

Contains 1 lg-like V-type (immunoglobulin-like) domain.

Post-translational

Polyubiquitinated; which is promoted by MARCH8 and results in endocytosis and lysosomal

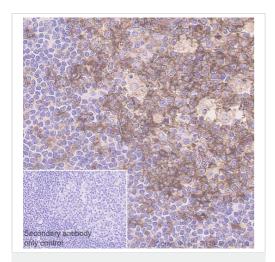
modifications

degradation.

Cellular localization

Membrane.

Images

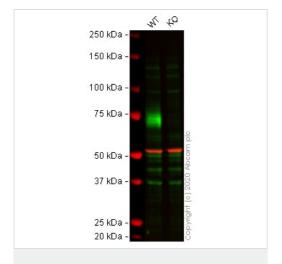


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD86 antibody [EP1158-37] (ab269587)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling CD86 with ab269587 at 1/100 dilution (2.52 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human tonsil (PMID: 22740445). The section was incubated with ab269587 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Western blot - Anti-CD86 antibody [EP1158-37] (ab269587)

All lanes : Anti-CD86 antibody [EP1158-37] (ab269587) at 1/1000 dilution

Lane 1 : Wild-type Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lane 2 : CD86 knockout Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 38 kDa
Observed band size: 70 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab269587 observed at 70 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab269587 was shown to react with CD86 in wild-type Raji cells in western blot with loss of signal observed in CD86 knockout sample. Wild-type and CD86 knockout Raji cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab269587 and ab7291 (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed

1 2 3

250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
37 kDa —
38 kDa —
39 kDa —
40 kDa —
40 kDa —
40 kDa —
41 kDa —
42 kDa —
43 kDa —
44 kDa —
45 kDa —
46 kDa —
47 kDa —
48 kDa —
48

Western blot - Anti-CD86 antibody [EP1158-37] (ab269587)

(<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-CD86 antibody [EP1158-37] (ab269587) at 1/1000 dilution

Lane 1 : Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate

Lane 2 : Daudi (human burkitt's lymphoma lymphoblast) whole cell lysate

Lane 3 : Jurkat (human t cell leukemia t lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 38 kDa Observed band size: 80 kDa

Blocking and dilution buffer: 5% NFDM/TBSt.

Exposure time: 3 minutes.

Negative control: Jurkat (PMID: 9973476).

The actual band size higher than predicted is mainly due to glycosylation.

The molecular weight observed is consistent with what has been described in the literature (PMID:27464357, 21896490).



Western blot - Anti-CD86 antibody [EP1158-37] (ab269587)

All lanes : Anti-CD86 antibody [EP1158-37] (ab269587) at 1/1000 dilution

Lane 1 : Human tonsil lysate
Lane 2 : Human spleen lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/1000 dilution

Predicted band size: 38 kDa Observed band size: 80 kDa

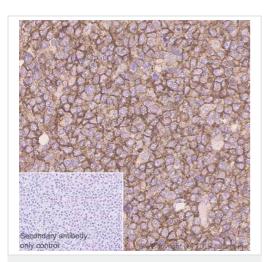
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

The actual band size higher than predicted is mainly due to glycosylation.

The molecular weight observed is consistent with what has been described in the literature (PMID:27464357, 21896490).

This blot was developed using a higher sensitivity ECL substrate.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD86 antibody [EP1158-37] (ab269587)

Immunohistochemical analysis of paraffin-embedded Human non-Hodgkin lymphoma tissue labeling CD86 with ab269587 at 1/100 dilution (2.52 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human non-Hodgkin lymphoma (PMID: 9767448,10089910). The section was incubated with ab269587 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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