abcam

Product datasheet

Anti-CD86 antibody [EPR21962] ab239075

KO VALIDATED Recombinant RabMAb

14 References 8 Images

Overview

Product name	Anti-CD86 antibody [EPR21962]
Description	Rabbit monoclonal [EPR21962] to CD86
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt, IP **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Ramos, Raji and Daudi whole cell lysates. ICC/IF: Raji and Daudi cells. Flow Cyt: Raji cells, Human monocyte-derived dendritic cells.
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: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR21962

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab239075 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).
ICC/IF		1/100.
Flow Cyt		1/500.
IP		1/30.

Application notes Is unsuitable for IHC-P.

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.

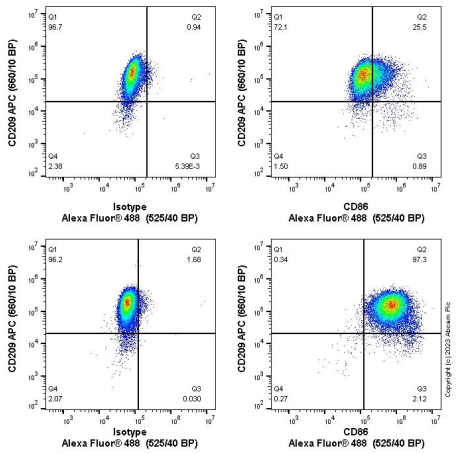
Tissue specificity Expressed by activated B-lymphocytes and monocytes.

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

Post-translational modifications Polyubiquitinated; which is promoted by MARCH8 and results in endocytosis and lysosomal degradation.

Cellular localization Membrane.

Images

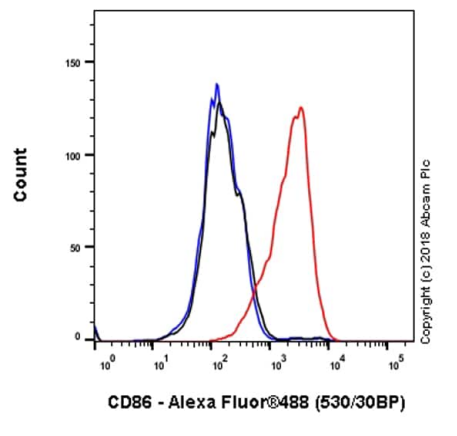


Flow Cytometry - Anti-CD86 antibody [EPR21962] (ab239075)

Flow cytometry staining of human monocyte-derived dendritic cells (top) or human monocyte-derived dendritic cells treated with 1µg/mL lipopolysaccharide (LPS) for 24h (bottom), with ab239075 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). Monocyte-derived dendritic cells were incubated for 30 min at 4°C in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab239075 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10^6 in 100µl at 0.04 µg/ml (1/56500)) for 30min on ice. The cells were simultaneously stained with CD209.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 4°C

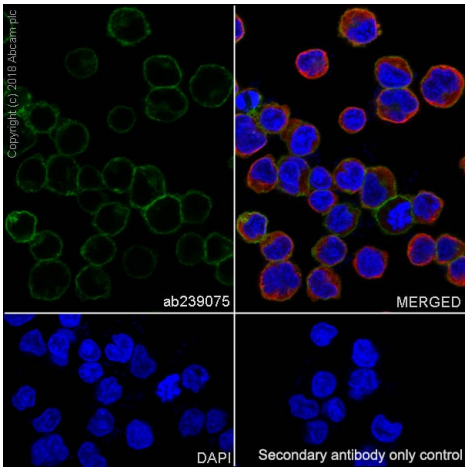
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on viable cells.



Flow Cytometry - Anti-CD86 antibody [EPR21962] (ab239075)

Flow cytometric analysis of Raji (human Burkitt's lymphoma cell line) cell line labeling CD86 with ab239075 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.

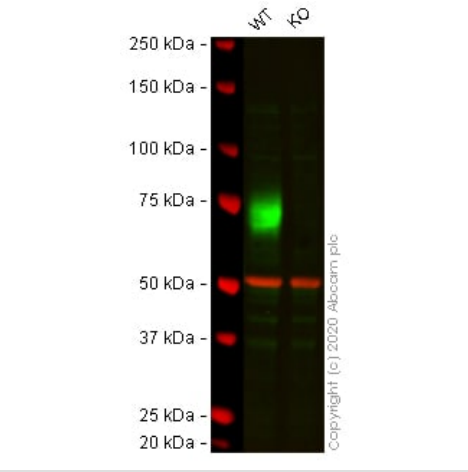


Immunocytochemistry/ Immunofluorescence - Anti-CD86 antibody [EPR21962] (ab239075)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Daudi (human Burkitt's lymphoma cell line) cells labeling CD86 with ab239075 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining in Daudi cell line is observed.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Western blot - Anti-CD86 antibody [EPR21962] (ab239075)

All lanes : Anti-CD86 antibody [EPR21962] (ab239075) 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.

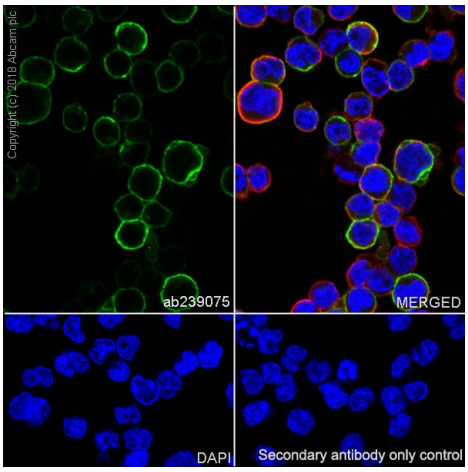
Performed under reducing conditions.

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

Lanes 1 - 2: Merged signal (red and green). Green - ab239075 observed at 70 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab239075 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 ab239075 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 IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

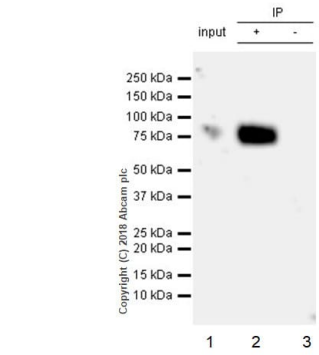


Immunocytochemistry/ Immunofluorescence - Anti-CD86 antibody [EPR21962] (ab239075)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raji (human Burkitt's lymphoma cell line) cells labeling CD86 with ab239075 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining in Raji cell line is observed.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-CD86 antibody [EPR21962] (ab239075)

CD86 was immunoprecipitated from 0.35 mg of Raji whole (human Burkitt's lymphoma cell line) cell lysate with ab239075 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab239075 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

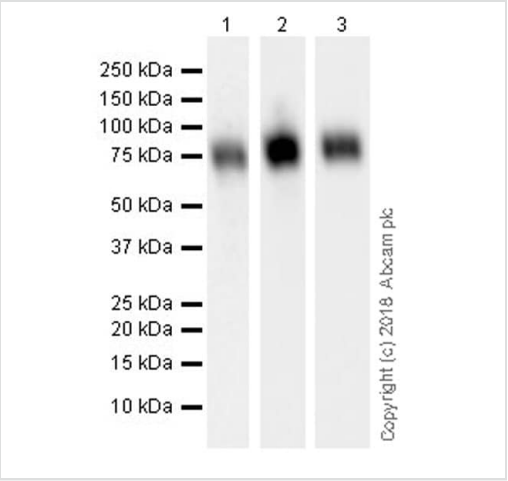
Lane 1: Raji whole cell lysate 10 µg (Input).

Lane 2: ab239075 IP in Raji whole lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab239075 in Raji whole cell lysate.

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

Exposure time: 3 minutes.



Western blot - Anti-CD86 antibody [EPR21962] (ab239075)

All lanes : Anti-CD86 antibody [EPR21962] (ab239075) at 1/1000 dilution

Lane 1 : Ramos (human Burkitt's lymphoma cell line) whole cell lysate
Lane 2 : Raji (human Burkitt's lymphoma cell line) whole cell lysate
Lane 3 : Daudi (human Burkitt's lymphoma cell line) whole cell lysate

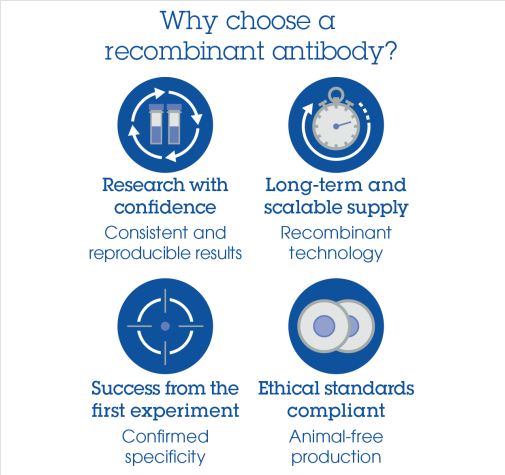
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time : Lane 1: 3 minutes; Lanes 2-3: 26 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.



Anti-CD86 antibody [EPR21962] (ab239075)

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