# abcam

# Product datasheet

# Anti-CD40 antibody [EPR18005-35] ab188181

Recombinant RabMAb

1 References 5 Images

Overview

**Product name** Anti-CD40 antibody [EPR18005-35]

**Description** Rabbit monoclonal [EPR18005-35] to CD40

**Host species** Rabbit

Specificity ab188181 recognises a different epitope to ab212058.

**Tested applications** Suitable for: WB, ICC/IF, Flow Cyt

Species reactivity Reacts with: Mouse

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

Positive control WB: A20 whole cell lysate. ICC/IF: A20 cells. Flow Cyt: A20 cells, C57 BL/6 mouse splenocytes.

**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 (glycerin, glycerine), 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal EPR18005-35 Clone number

Isotype lgG

#### **Applications**

## The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab188181 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 32-42 kDa (predicted molecular weight: 30 kDa).
ICC/IF		1/100.
Flow Cyt		1/500.

# **Target**

**Function** Receptor for TNFSF5/CD40LG.

**Tissue specificity** B-cells and in primary carcinomas.

**Involvement in disease**Defects in CD40 are the cause of hyper-lgM immunodeficiency syndrome type 3 (HIGM3)

[MIM:606843]; also known as hyper-lgM syndrome 3. HIGM3 is an autosomal recessive disorder which includes an inability of B cells to undergo isotype switching, one of the final differentiation steps in the humoral immune system, an inability to mount an antibody-specific immune response,

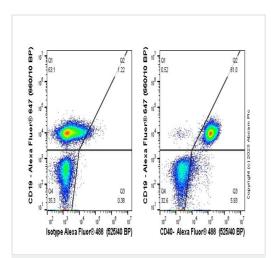
and a lack of germinal center formation.

Contains 4 TNFR-Cys repeats.

Sequence similarities

**Cellular localization** Secreted and Cell membrane.

#### **Images**

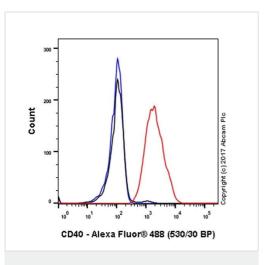


Flow Cytometry - Anti-CD40 antibody [EPR18005-35] (ab188181)

Flow cytometry staining of C57 BL/6 mouse splenocytes with ab188181 (right) or Recombinant Rabbit lgG, monoclonal [EPR25A] - Isotype Control (left). Cells were incubated for 30 min on ice in 1x PBS containing 10 $\mu$ g/ml anti CD16/CD32 and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab188181 or Recombinant Rabbit lgG, monoclonal [EPR25A] - Isotype Control (1x 10<sup>6</sup> in 100  $\mu$ l at 5.0  $\mu$ g/ml (1/388)) for 30min on ice. The cells were simultaneously stained with CD19.

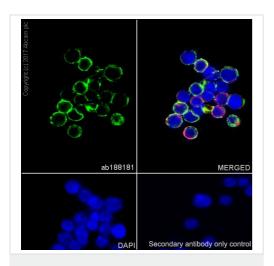
The secondary antibody Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice

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-CD40 antibody [EPR18005-35] (ab188181)

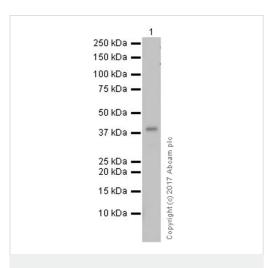
Flow cytometric analysis of A20 (mouse reticulum sarcoma cell line) cell line labeling CD40 with ab188181 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-CD40 antibody [EPR18005-35] (ab188181)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A20 (mouse reticulum sarcoma cell line) cells labeling CD40 with ab188181 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining on A20 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (<u>ab195889</u>) at 1/200 dilution (red).



Western blot - Anti-CD40 antibody [EPR18005-35] (ab188181)

Anti-CD40 antibody [EPR18005-35] (ab188181) at 1/1000 dilution + A20 (mouse reticulum sarcoma cell line) whole cell lysate at 10 µg

### Secondary

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

Developed using the ECL technique.

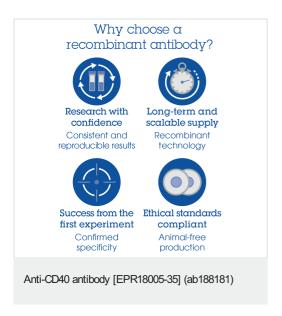
Performed under reducing conditions.

**Predicted band size:** 30 kDa **Observed band size:** 32-42 kDa

Blocking/Dilution: 5% NFDM/TBST.

Exposure: 15 seconds.

The expression profile observed is consistent with what has been described in the literature (PMID: 23404288 and PMID: 16709811).



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