abcam

ab228818

Anti-CD40 antibody [EPR20735] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR20735
Purification technique	Affinity purification Protein A
Concentration	0.967 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	-

WB

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/500
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information CD40

Function	Receptor for TNFSF5/CD40LG (PubMed:31331973). Transduces TRAF6- and MAP3K8-mediated signals that activate ERK in macrophages and B cells, leading to induction of immunoglobulin secretion (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab228818 is the carrier-free version of ab224639.

Patented technology

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

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based 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.

Compatibility

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

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

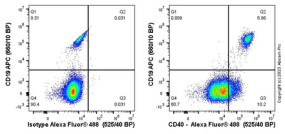
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

9 product images



Flow Cytometry - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

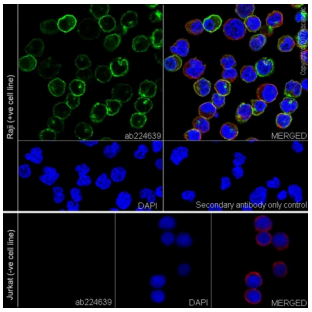
CD40 Flow Cytometry staining using rabbit Anti-CD40 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224639).

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab224639 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). PBMCs were incubated for 30 min on ice 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 ab224639 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 106 in 100 µl at 0.2 µg/ml (1/10550)) for 30min on ice. The cells were simultaneously stained with CD19.

The secondary antibody Goat Anti-Rabbit IgG 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.



Immunocytochemistry/ Immunofluorescence - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

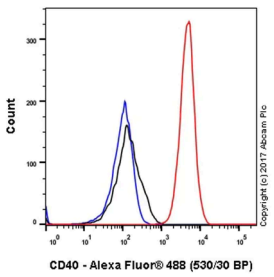
CD40 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD40 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raji (human Burkitt's lymphoma cell line) cells labeling CD40 with ab224639 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 and weakly cytoplasmic staining in Raji cell line.
Negative control: Jurkat T cell line (PMID:10498643).

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

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224639).

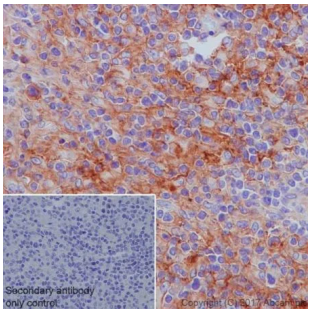


Flow Cytometry - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

CD40 Flow Cytometry staining using rabbit Anti-CD40 antibody

Flow cytometric analysis of Raji (human Burkitt's lymphoma cell line) cell line labeling CD40 with ab224639 at 1/600 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 total viable cells.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224639).



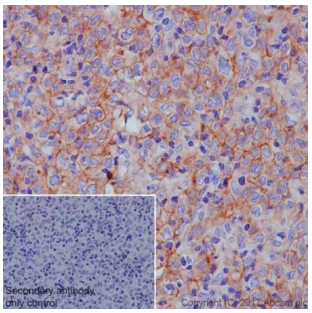
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

CD40 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD40 antibody

Immunohistochemical analysis of paraffin-embedded human Hodgkin's lymphoma tissue labeling CD40 with ab224639 at 1/250 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Membranous and cytoplasmic staining in Reed-Sternberg cells and tumor cells of human Hodgkin's lymphoma (PMID:7507299) is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224639).



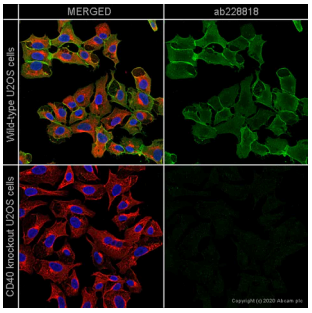
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

CD40 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD40 antibody

Immunohistochemical analysis of paraffin-embedded human diffuse large B-cell lymphoma tissue labeling CD40 with ab224639 at 1/250 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Membranous staining in human diffuse large B-cell lymphoma tissue (PMID: 20593977) is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

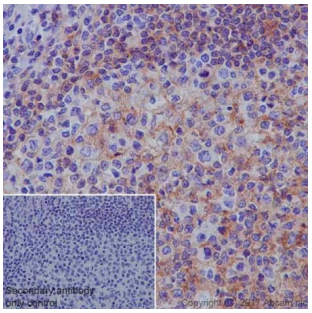
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224639).



Immunocytochemistry/ Immunofluorescence - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

CD40 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD40 antibody

ab228818 staining CD40 in wild-type U2OS cells (top panel) and CD40 knockout U2OS cells (bottom panel). The cells were fixed with PFA (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab228818 at 1/100 dilution and ab7291 (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



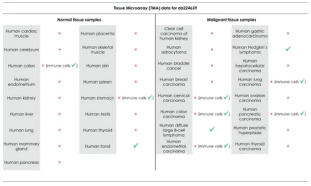
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

CD40 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD40 antibody

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD40 with ab224639 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and cytoplasmic staining in human tonsil tissue (PMID:20593977; PMID:7507299) is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

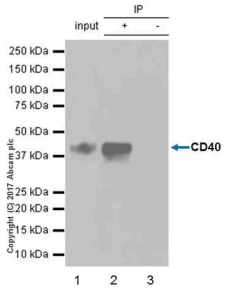
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224639).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

Tissue Microarrays stained for "Anti-CD40 antibody [EPR20735]" using "ab224639"in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab224639 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



Immunoprecipitation - Anti-CD40 antibody [EPR20735] - BSA and Azide free (ab228818)

CD40 was immunoprecipitated from 0.35 mg of Raji (human Burkitt's lymphoma cell line) whole cell lysate with ab224639 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab224639 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.
Lane 1: Raji whole cell lysate 10 µg (Input).
Lane 2: ab224639 IP in Raji whole cell lysate (+).
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab224639 in Raji whole cell lysate (-).

Exposure time: 3 minutes.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab224639).

All lanes:
Immunoprecipitation - Anti-CD40 antibody [EPR20735] (ab224639)

Developed using the ECL technique.

Predicted band size: 30 kDa

Observed band size: 42 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.