

Product datasheet

Anti-CD40 antibody [EPR20735] ab224639

KO VALIDATED Recombinant RabMAb

6 References 9 Images

Overview

Product name	Anti-CD40 antibody [EPR20735]
Description	Rabbit monoclonal [EPR20735] to CD40
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, Flow Cyt, IP, ICC/IF
Species reactivity	Reacts with: Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human lymph node and lymphoma tissue lysates; Raji whole cell lysate. IHC-P: Human tonsil, Hodgkin's lymphoma and diffuse large B-cell lymphoma tissues. ICC/IF: Raji cells. Flow Cyt: Raji cells. IP: Raji whole cell lysate. ICC/IF KO: U2OS cells (U2OS-CD40 knockout cells used as negative cell line)
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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
Clone number	EPR20735

Isotype

IgG

Applications

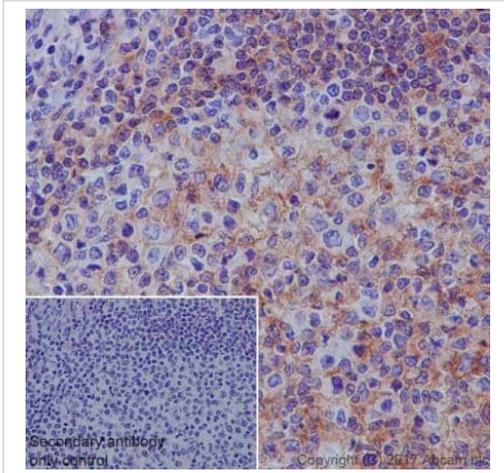
The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab224639 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 42 kDa (predicted molecular weight: 30 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		1/600. ab172730 -Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		1/30.
ICC/IF		1/100.

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-IgM immunodeficiency syndrome type 3 (HIGM3) [MIM:606843]; also known as hyper-IgM 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.
Sequence similarities	Contains 4 TNFR-Cys repeats.
Cellular localization	Secreted and Cell membrane.

Images

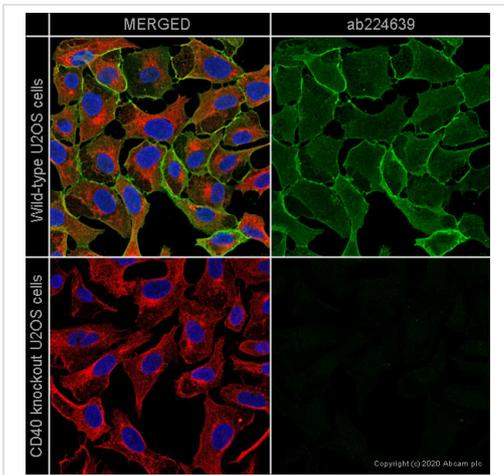


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] (ab224639)

Immunohistochemical analysis of paraffin-embedded human tonsil 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 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 a ready to use Goat Anti-Rabbit IgG H&L (HRP).

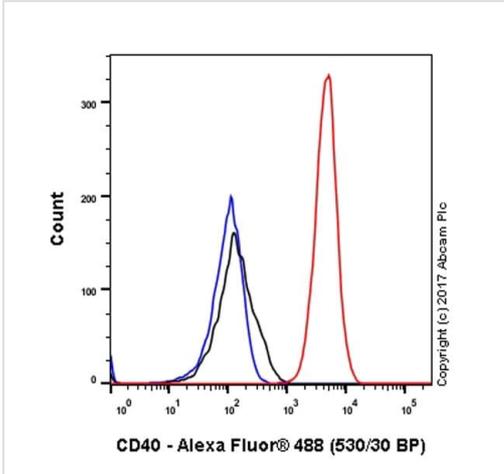
Perform heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-CD40 antibody [EPR20735] (ab224639)

ab224639 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 ab224639 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.

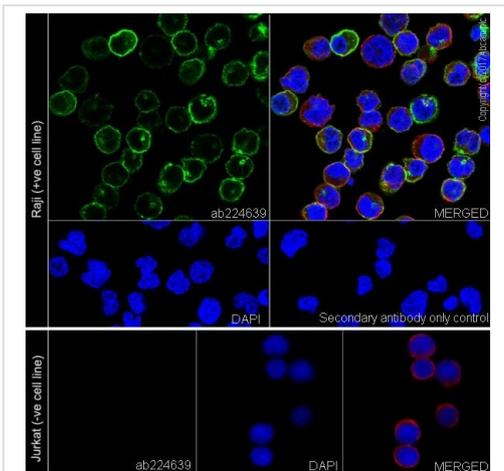
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry - Anti-CD40 antibody [EPR20735] (ab224639)

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.



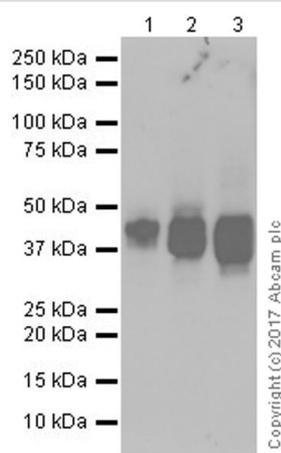
Immunocytochemistry/ Immunofluorescence - Anti-CD40 antibody [EPR20735] (ab224639)

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.



Western blot - Anti-CD40 antibody [EPR20735]
(ab224639)

All lanes : Anti-CD40 antibody [EPR20735] (ab224639) at 1/1000 dilution

Lane 1 : Human lymph node tissue lysate

Lane 2 : Human lymphoma tissue lysate

Lane 3 : Raji (human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

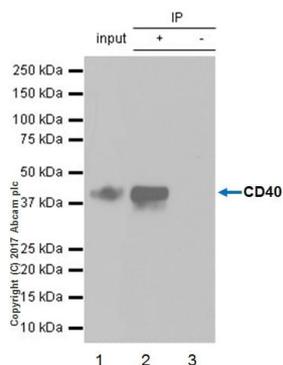
Developed using the ECL technique.

Predicted band size: 30 kDa

Observed band size: 42 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.



Immunoprecipitation - Anti-CD40 antibody
[EPR20735] (ab224639)

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.

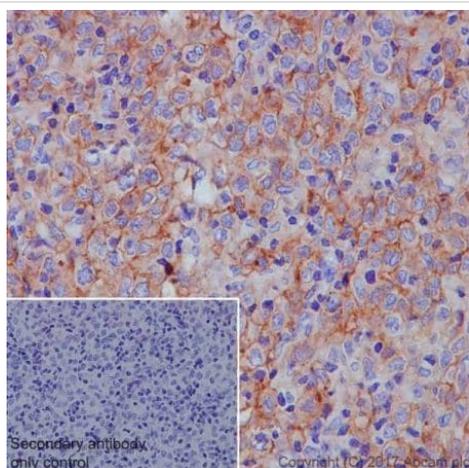
Lane 1: Raji whole cell lysate 10 µg (Input).

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.

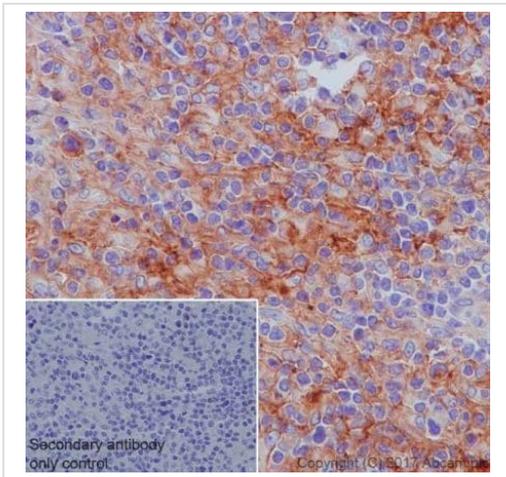


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody
[EPR20735] (ab224639)

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).



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).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] (ab224639)

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-CD40 antibody [EPR20735] (ab224639)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors