abcam

Product datasheet

Anti-CD40 antibody ab113701



3 Images

Overview

Product name Anti-CD40 antibody

Description Rabbit polyclonal to CD40

Host species Rabbit

Tested applications Suitable for: WB, IHC-P Species reactivity Reacts with: Human

Predicted to work with: Macaque monkey, Gorilla

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

Positive control WB: U-2 OS and Raji cell lysates; Human spleen and thymus tissue lysates. IHC-FFPE: Human

spleen tissue.

General notes 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 or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

Purity Immunogen affinity purified

1

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab113701 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		Use a concentration of 1 µg/ml. Detects a band of approximately 43 kDa (predicted molecular weight: 31 kDa).
IHC-P		Use a concentration of 5 µg/ml.

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)

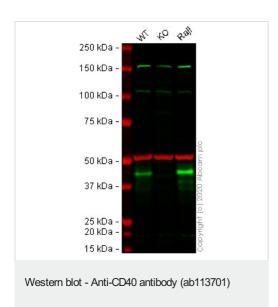
[MIM:606843]; also known as hyper-IgM syndrome 3. HIGM3 is an autosomal recessive disorder which includes an inability of B cells to undergo isotype switching, one of the final differentiation steps in the humoral immune system, an inability to mount an antibody-specific immune response,

and a lack of germinal center formation.

Sequence similarities Contains 4 TNFR-Cys repeats.

Cellular localization Secreted and Cell membrane.

Images



All lanes: Anti-CD40 antibody (ab113701) at 1 µg/ml

Lane 1: Wild-type U-2 OS cell lysate

Lane 2: CD40 knockout U-2 OS cell lysate

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

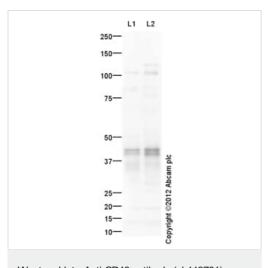
Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Predicted band size: 31 kDa **Observed band size:** 45 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab113701 observed at 45 kDa. Red - loading control, <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab113701 was shown to react with CD40 in wild-type U-2 OS cells in Western blot. Loss of signal was observed when CD40 knockout cell line ab262486 (knockout cell lysate ab263923) was used. Wild-type and CD40 knockout U-2 OS cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab113701 and ab7291 (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-CD40 antibody (ab113701)

All lanes: Anti-CD40 antibody (ab113701) at 1 µg/ml

Lane 1 : Human spleen tissue lysate - total protein (<u>ab29699</u>)

Lane 2 : Human thymus tissue lysate - total protein (ab30146)

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

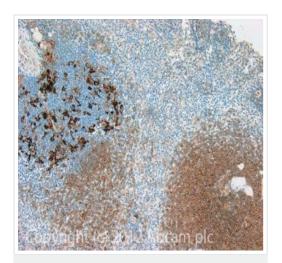
Predicted band size: 31 kDa **Observed band size:** 43 kDa

Additional bands at: 105 kDa, 41 kDa (possible cleavage fragment). We are unsure as to the identity of these extra bands.

Exposure time: 10 minutes

CD40 contains a number of potential glycosylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted. The predicted molecular weight of CD40 is

31 kDa (SwissProt), however we expect to observe a banding pattern around 43 kDa. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD40 antibody (ab113701)

IHC image of CD40 staining in Human normal spleen formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab113701, $5\mu g/ml$, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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

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