abcam

Product datasheet

Anti-CD40 antibody [EPR20540] - Low endotoxin, Azide free ab223546

KO VALIDATED Recombinant RabMAb

6 Images

Overview

Product name	Anti-CD40 antibody [EPR20540] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR20540] to CD40 - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human tonsil, lymph node and lymphoma lysates; Raji, U-2 OS, and Daudi whole cell lysates. IHC-P: Human tonsil and large B cell lymphoma tissues. IMC: Human tonsil tissue.
General notes	ab223546 is the carrier-free version of **ab213205**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20540
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab223546 in the following tested applications.

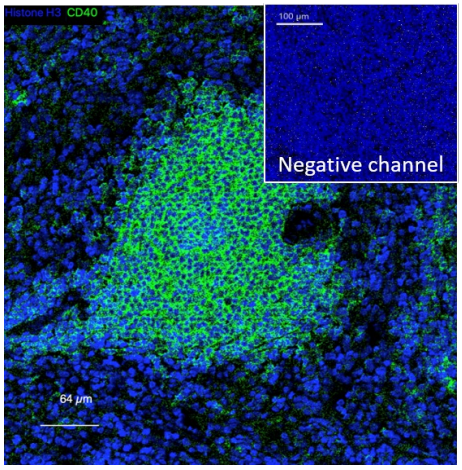
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 42 kDa (predicted molecular weight: 30 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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

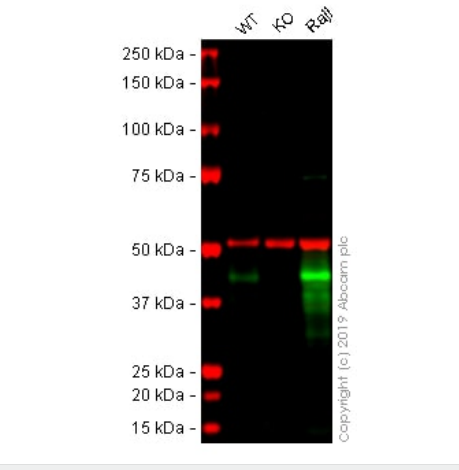


Mass Cytometry - Anti-CD40 antibody [EPR20540] - Low endotoxin, Azide free (ab223546)

This image is courtesy of the Single Cell & Imaging Mass Cytometry Analysis Platform, Goodman Cancer Research Centre, McGill University

Imaging Mass Cytometry™ (IMC™) image of human tonsil tissue stained with Anti-CD40 antibody [EPR20540]. ab223546 (carrier-free antibody, purified) was metal-conjugated using a Maxpar® Antibody Labeling Kit from Fluidigm. Immunostaining was performed according to Fluidigm's protocols. Briefly, slides were subject to deparaffinization and heat-induced epitope retrieval, followed by overnight incubation at 4°C with an antibody cocktail containing metal-tagged antibodies in blocking buffer. Slides were subsequently washed with 0.2% Triton-X and 1x PBS, counterstained with Cell-ID™ Intercalator-Ir diluted at 1/400 in 1x PBS for 30 min at room temperature, rinsed for 5 min with distilled H2O, and air-dried prior to IMC™ acquisition. IMC™ acquisition was performed using the Fluidigm Hyperion™ Imaging System.

Imaging Mass Cytometry™, IMC™, Cell-ID™, Hyperion™ and Maxpar® are trademarks of Fluidigm Canada



Western blot - Anti-CD40 antibody [EPR20540] - Low endotoxin, Azide free (ab223546)

All lanes : Anti-CD40 antibody [EPR20540] (**ab213205**) at 1/2000 dilution

Lane 1 : Wild-type U-2 OS whole cell lysate
Lane 2 : CD40 knockout U-2 OS whole cell lysate
Lane 3 : Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

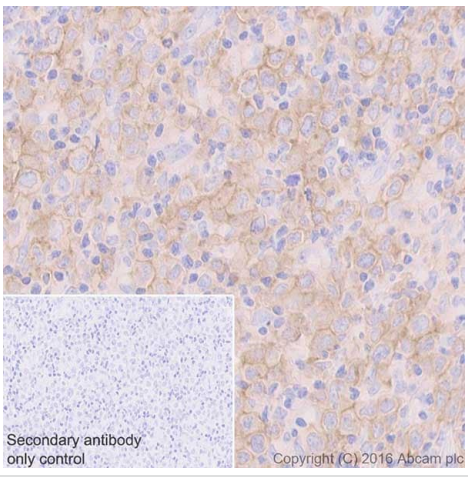
Predicted band size: 30 kDa
Observed band size: 42 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab213205**).

Lanes 1 - 3: Merged signal (red and green). Green - **ab213205** observed at 42 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab213205 was shown to react with CD40 in U-2 OS wild-type cells

in Western blot. Loss of signal was observed when CD40 knockout sample was used. U-2 OS wild-type and CD40 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with **ab213205** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were developed 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20540] - Low endotoxin, Azide free (ab223546)

Immunohistochemical analysis of paraffin-embedded human large B cell lymphoma tissue labeling CD40 with **ab213205** at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

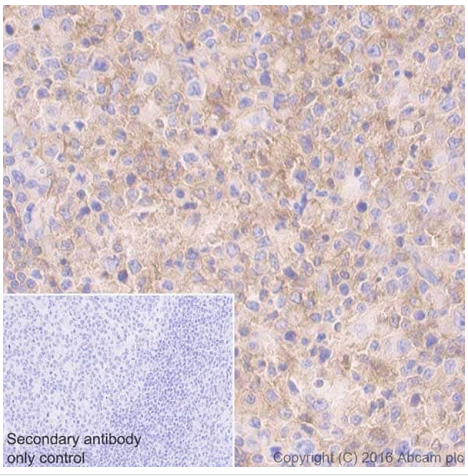
Membranous staining on human large B cell lymphoma is observed [PMID: 7507299].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213205**).

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 [EPR20540] - Low endotoxin, Azide free (ab223546)

This IHC data was generated using the same anti-CD40 antibody clone [EPR20540] in a different buffer formulation (cat# **ab213205**).

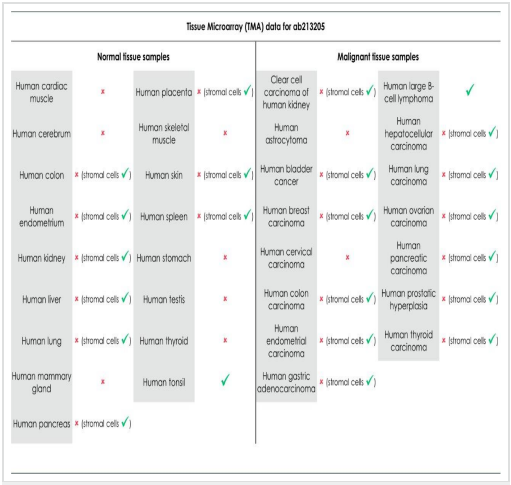
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD40 with **ab213205** at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Membranous and cytoplasmic staining on germinal center of human tonsil is observed [PMID: 10360965] [PMID: 7507299] [PMID:24452203].

Counter stained with Hematoxylin.

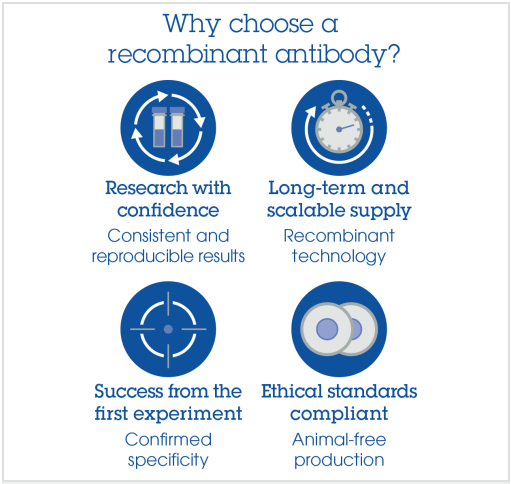
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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 [EPR20540] - Low endotoxin, Azide free (ab223546)

Tissue Microarrays stained for " Anti-CD40 antibody [EPR20540]" using " **ab213205**" 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 **ab213205** at +4°C overnight followed by Goat Anti-Rabbit IgG H&L (HRP) **ab97051** at 1/500.



Anti-CD40 antibody [EPR20540] - Low endotoxin, Azide free (ab223546)

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