abcam

Product datasheet

Human CD40 knockout U-2 OS cell lysate ab263923

4 Images

Overview

Product name	Human CD40 knockout U-2 OS cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	U-2 OS
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion, 2 bp insertion; Frameshift: 99.09%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

Properties

Storage instructions	Store at -80°C. Please refer to protocols.

Components	1 kit
ab280476 - Human CD40 knockout U2OS cell lysate	1 x 100µg
ab263974 - Human wild-type U-2 OS cell lysate	1 x 100µg

Cell type	epithelial
Disease	Osteosarcoma
Gender	Female

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.

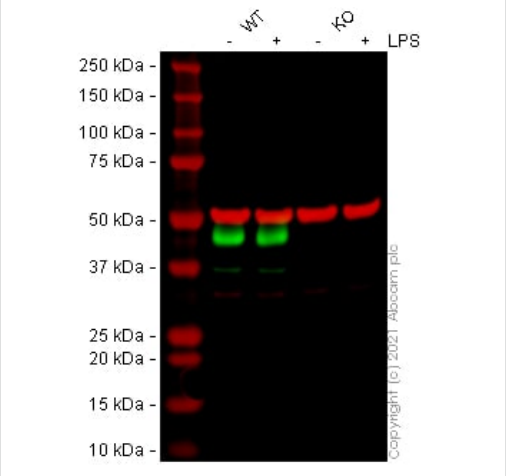
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab263923 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. Predicted molecular weight: 30 kDa.

Images



Western blot -Human CD40 knockout U-2 OS cell lysate

Lane 1: Wild-type U-2 OS Vehicle Control LPS (0µg/mL, 6h) cell lysate 20 µg

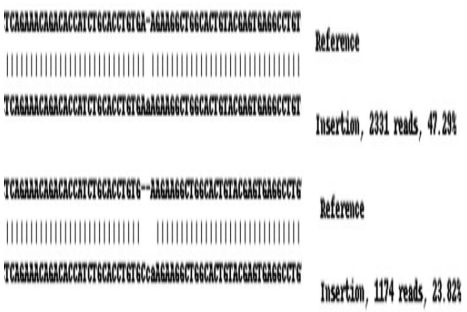
Lane 2: Wild-type U-2 OS Treated LPS (1µg/mL, 6h) cell lysate 20 µg

Lane 3: CD40 knockout U-2 OS Vehicle Control LPS (0µg/mL, 6h) cell lysate 20 µg

Lane 4: CD40 knockout U-2 OS Treated LPS (1µg/mL, 6h) cell lysate 20 µg

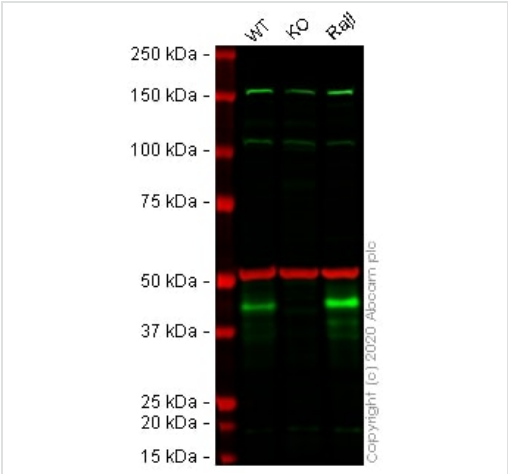
Lanes 1 - 4: Merged signal (red and green). Green - **ab280207** observed at 45 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab280207 was shown to react with CD40 in wild-type U-2 OS cells in Western blot with loss of signal observed in CD40 knockout cell line **ab262486** (CD40 knockout cell lysate ab263923). Wild-type U-2 OS 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 **ab280207** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 h t room temperature before imaging.



Next Generation Sequencing - Human CD40 knockout U-2 OS cell lysate (ab263923)

Knockout achieved by CRISPR/Cas9; X = 1 bp insertion, 2 bp insertion; Frameshift: 99.09%



Western blot - Human CD40 knockout U-2 OS cell lysate (ab263923)

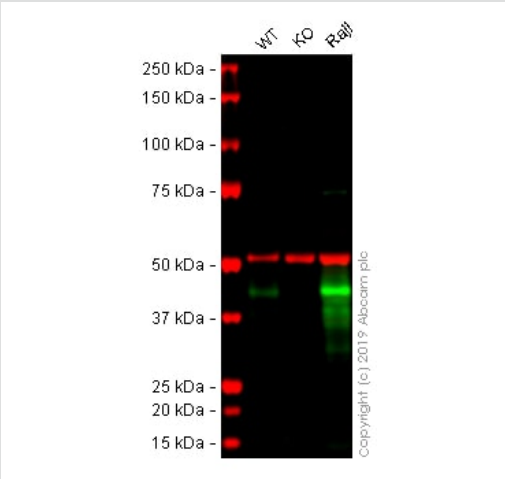
Lane 1: Wild-type U-2 OS cell lysate

Lane 2: CD40 knockout U-2 OS cell lysate

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

Lanes 1 - 3: Merged signal (red and green). Green - **ab113701** observed at 45 kDa. Red - loading control, **ab7291** (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 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.



Western blot - Human CD40 knockout U-2 OS cell lysate (ab263923)

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

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 cell line **ab262486** (knockout cell lysate ab263923) was used. Wild-type U-2 OS 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.

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