abcam

Product datasheet

Human CD40 knockout U-2 OS cell line ab262486

6 Images

Overview

Product name	Human CD40 knockout U-2 OS cell line
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	Immunocytochemistry (ICC), Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB, ICC
Biosafety level	1
General notes	**Recommended control:** Human wild-type U-2 OS cell line (**ab263976**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** McCoY5a + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.
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We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Bone
Cell type	epithelial
Disease	Osteosarcoma
Gender	Female
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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.

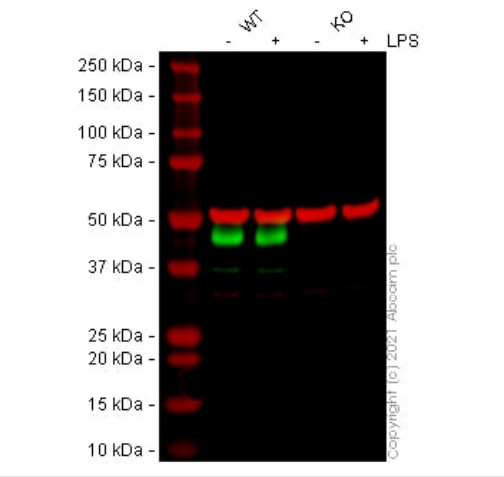
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab262486 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.
ICC		Use at an assay dependent concentration.

Images



Western blot - Human CD40 knockout U-2 OS cell line (ab262486)

All lanes : Anti-CD40 antibody [41/CD40] (**ab280207**) at 1/1000 dilution

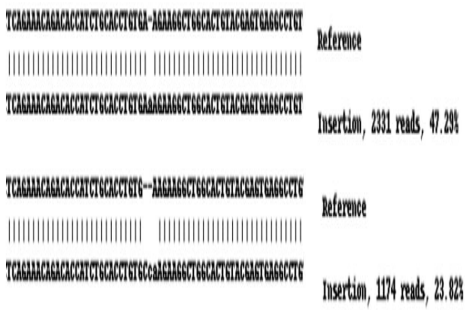
Lane 1 : Wild-type U-2 OS Vehicle Control LPS (0µg/mL, 6h) cell lysate
Lane 2 : Wild-type U-2 OS Treated LPS (1µg/mL, 6h) cell lysate
Lane 3 : CD40 knockout U-2 OS Vehicle Control LPS (0µg/mL, 6h) cell lysate
Lane 4 : CD40 knockout U-2 OS Treated LPS (1µg/mL, 6h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

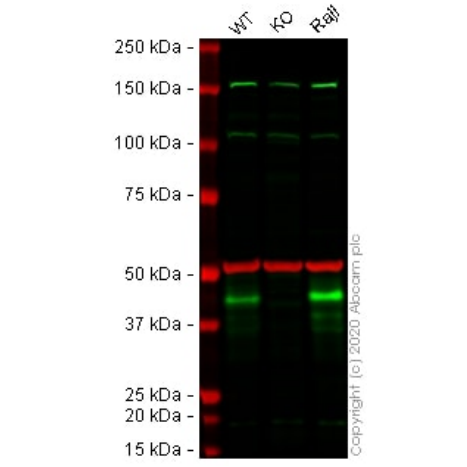
Lanes 1 - 4: Merged signal (red and green). Green - **ab280207** observed at 45 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

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 at room temperature before imaging.



Next Generation Sequencing - Human CD40 knockout U-2 OS cell line (ab262486)

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



Western blot - Human CD40 knockout U-2 OS cell line (ab262486)

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

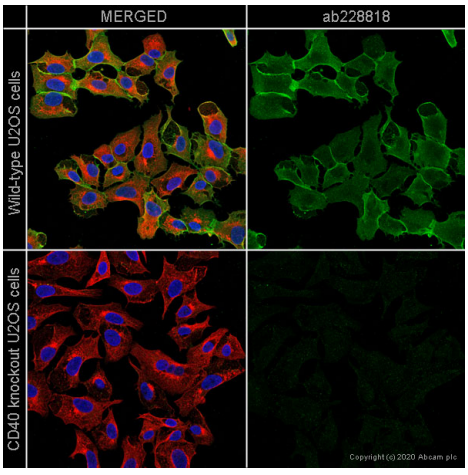
Lane 1 : Wild-type U-2 OS cell lysate
Lane 2 : CD40 knockout U-2 OS cell lysate
Lane 3 : Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

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.

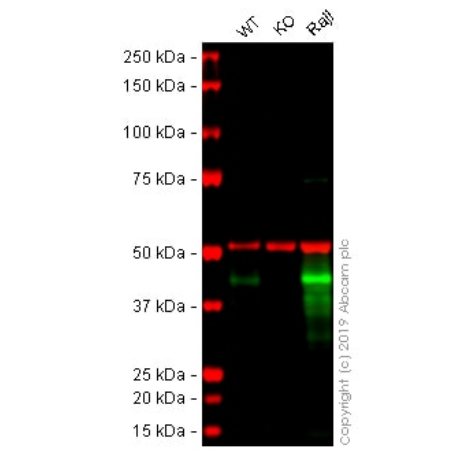
ab113701 was shown to react with CD40 in wild-type U-2 OS cells in Western blot Loss of signal was observed when CD40 knockout cell line ab262486 (knockout cell lysate **ab263923**) was used. Wild-type and CD40 knockout U-2 OS cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk 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.



Immunocytochemistry/ Immunofluorescence - Human CD40 knockout U-2 OS cell line (ab262486)

ab228818 staining CD40 in wild-type U-2 OS cells (top panel) and CD40 knockout U-2 OS cells (ab262486) (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).



Western blot - Human CD40 knockout U-2 OS cell line (ab262486)

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

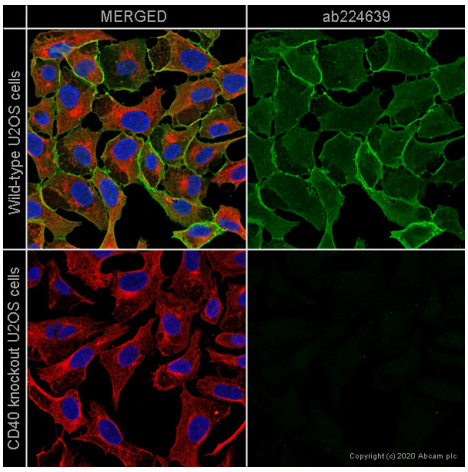
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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.



Immunocytochemistry/ Immunofluorescence - Human CD40 knockout U-2 OS cell line (ab262486)

ab224639 staining CD40 in wild-type U-2 OS cells (top panel) and CD40 knockout U-2 OS cells (ab262486) (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).

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