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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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
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.

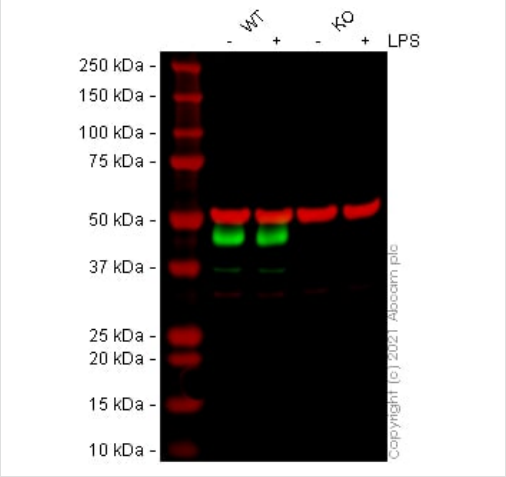
Applications

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)

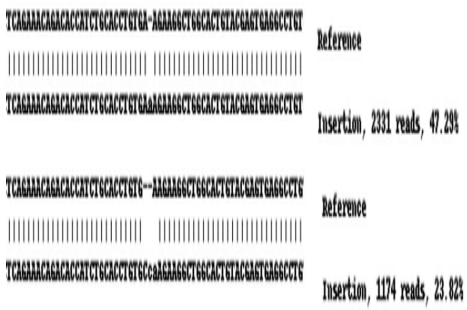
All lanes : Anti-CD40 antibody [41/CD40] (ab280207) at 1/1000 dilution

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.

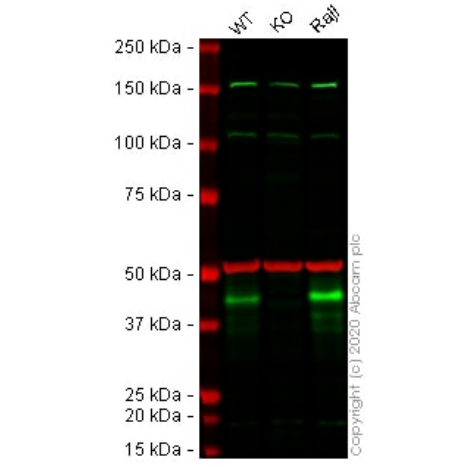
Lanes 1 - 4: Merged signal (red and green). Green - ab280207 observed at 45 kDa. Red - loading control ab52866 (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

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)

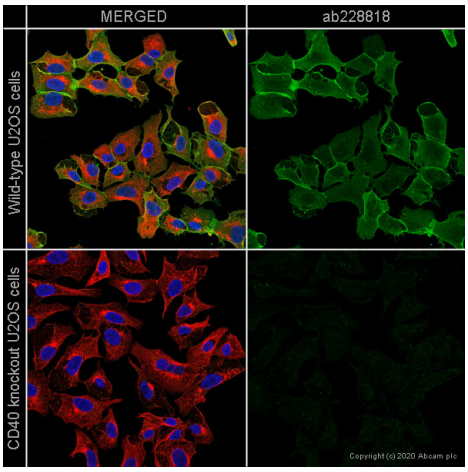
All lanes : Anti-CD40 antibody (ab113701) at 1 µg/ml

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

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.

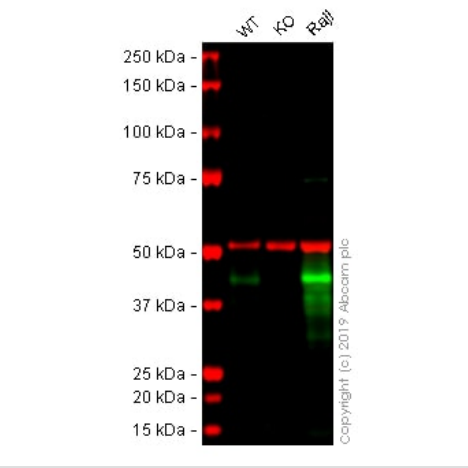
ab113701 was shown to react with CD40 in wild-type U-2 OS cells in Western blot Loss of signal was observed when CD40 knockout cell line ab262486 (knockout cell lysate ab263923) was used. Wild-type and CD40 knockout U-2 OS cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in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

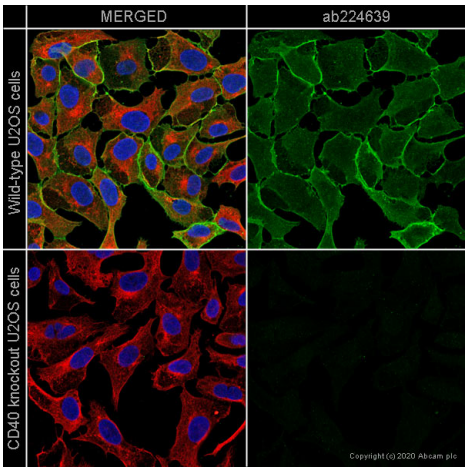
Lane 1 : Wild-type U-2 OS cell lysate
Lane 2 : CD40 knockout U-2 OS whole cell lysate
Lane 3 : Raji (Human Burkitt's lymphoma cell line) whole cell lysate

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 - 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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