abcam

Product datasheet

Human CD47 knockout HEK-293T cell line ab266324

6 Images

Overview

Product name	Human CD47 knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 11 bp deletion in exon 2 and 5 bp deletion in exon 2
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** ICC, WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK293T cell line (**ab255449**). 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:** DMEM (High Glucose) + 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	Kidney
Cell type	epithelial
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
Antibiotic resistance	Puromycin 1.00µg/ml
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	Has a role in both cell adhesion by acting as an adhesion receptor for THBS1 on platelets, and in the modulation of integrins. Plays an important role in memory formation and synaptic plasticity in the hippocampus (By similarity). Receptor for SIRPA, binding to which prevents maturation of immature dendritic cells and inhibits cytokine production by mature dendritic cells. Interaction with SIRPG mediates cell-cell adhesion, enhances superantigen-dependent T-cell-mediated proliferation and costimulates T-cell activation. May play a role in membrane transport and/or integrin dependent signal transduction. May prevent premature elimination of red blood cells. May be involved in membrane permeability changes induced following virus infection.
Tissue specificity	Very broadly distributed on normal adult tissues, as well as ovarian tumors, being especially abundant in some epithelia and the brain.
Sequence similarities	Contains 1 Ig-like V-type (immunoglobulin-like) domain.
Cellular localization	Cell membrane.

Applications

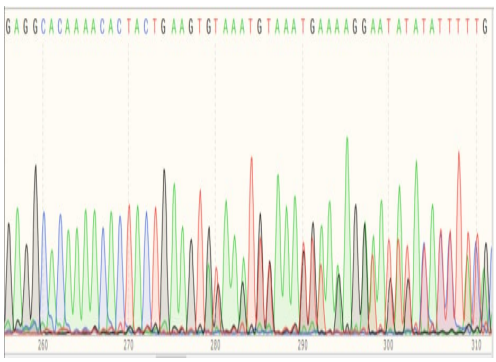
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266324 in the following tested applications.

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

Application	Abreviews	Notes
ICC		Use at an assay dependent concentration.

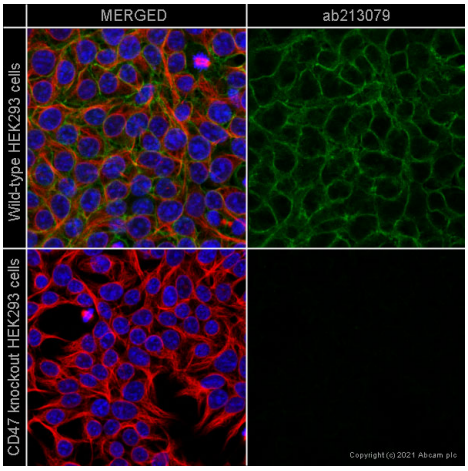
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 35 kDa.

Images



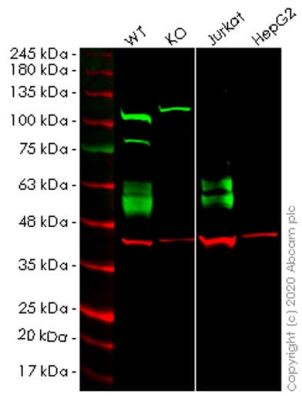
Sanger Sequencing - Human CD47 knockout HEK-293T cell line (ab266324)

Sequencing chromatogram displaying sequence edit in exon 2



Immunocytochemistry - Human CD47 knockout HEK-293T cell line (ab266324)

ab213079 staining CD47 in wild-type HEK293 cells (top panel) and CD47 knockout HEK293 cells (bottom panel) (ab266324). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Tween 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 **ab213079** at 0.4 µg/ml concentration and **ab6046** (Rabbit polyclonal to beta 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 mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Human CD47 knockout HEK293T cell line (ab266324)

All lanes : Anti-CD47 antibody [EPR21794] (**ab218810**) at 1/500 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : CD47 knockout HEK293T cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

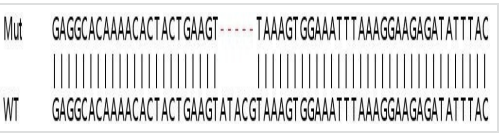
Predicted band size: 35 kDa
Observed band size: 47-52 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab218810** observed at 47-52 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab218810 Anti-CD47 antibody [EPR21794] was shown to specifically react with CD47 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266324 (knockout cell lysate **ab257220**) was used. Wild-type and CD47 knockout samples were subjected to SDS-PAGE. **ab218810** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

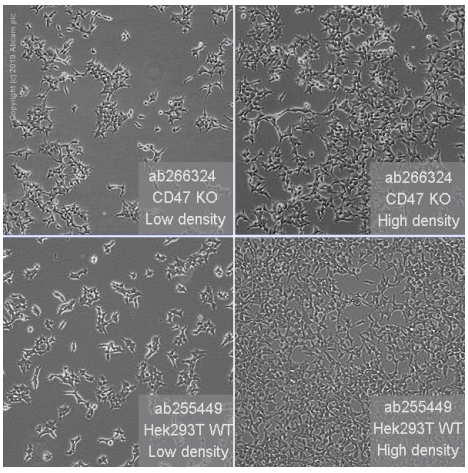
Sanger Sequencing - Human CD47 knockout HEK293T cell line (ab266324)

Allele-1: 11 bp deletion in exon 2



Sanger Sequencing - Human CD47 knockout HEK293T cell line (ab266324)

Allele-2: 5 bp deletion in exon 2.



Cell Culture - Human CD47 knockout HEK293T cell line (ab266324)

Representative images of CD47 knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS XL Core microscope.

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