

Product datasheet

Human CD58 knockout HeLa cell line ab265947

4 Images

Overview

Product name	Human CD58 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 11 bp deletion in exon 1 and 2 bp deletion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Recommended control: Human wild-type HeLa cell line (ab255928). 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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

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	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

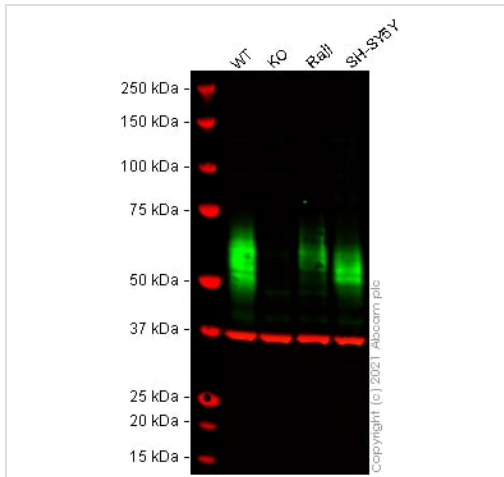
Function	Ligand of the T-lymphocyte CD2 glycoprotein. This interaction is important in mediating thymocyte interactions with thymic epithelial cells, antigen-independent and -dependent interactions of T-lymphocytes with target cells and antigen-presenting cells and the T-lymphocyte rosetting with erythrocytes. In addition, the LFA-3/CD2 interaction may prime response by both the CD2+ and LFA-3+ cells.
Sequence similarities	Contains 1 Ig-like C2-type (immunoglobulin-like) domain.
Cellular localization	Cell membrane.

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab265947 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: 28 kDa.

Images



Western blot - Human CD58 knockout HeLa cell line (ab265947)

All lanes : Anti-CD58 antibody [EPR24012-147] ([ab275392](#)) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : CD58 knockout HeLa cell lysate

Lane 3 : Raji cell lysate

Lane 4 : SH-SY5Y cell lysate

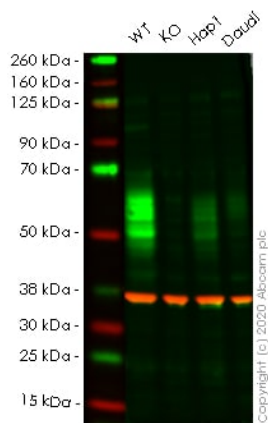
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 28 kDa

Observed band size: 55 kDa

False colour image of Western blot: Anti-CD58 antibody [EPR24012-147] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] ([ab8245](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, [ab275392](#) was shown to bind specifically to CD58. A band was observed at 55 kDa in wild-type HeLa cell lysates with no signal observed at this size in CD58 knockout cell line ab265947 (knockout cell lysate [ab257880](#)). To generate this image, wild-type and CD58 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween[®] 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.



Western blot - Human CD58 knockout HeLa cell line (ab265947)

All lanes : Anti-CD58 antibody [EP15041] ([ab196648](#)) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : CD58 knockout HeLa cell lysate

Lane 3 : Wild-type HAP1 cell lysate

Lane 4 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/20000 dilution

Predicted band size: 28 kDa

Observed band size: 43 kDa

Lanes 1-4: Merged signal (red and green). Green - [ab196648](#) observed at 43 kDa. Red - loading control [ab8245](#) observed at 37 kDa.

[ab196648](#) Anti-CD58 antibody [EP15041] was shown to specifically react with CD58 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265947 (knockout cell lysate [ab257880](#)) was used. Wild-type and CD58 knockout samples were subjected to SDS-PAGE. [ab196648](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4°C at 1 in 1000 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.

Mut	CGGCCCGACGAGCCATGGTTGCTGGGA-----CGGGCCCTGGGGTCTCAGCG
WT	CGGCCCGACGAGCCATGGTTGCTGGGAGCGACGCGGGGCGGGCCCTGGGGTCTCAGCG

Allele-1: 11 bp deletion in exon 1.

Sanger Sequencing - Human CD58 knockout HeLa cell line (ab265947)

Mut	CGGCCCGACGAGCCATGGTTGCTGGGA--GACGCGGGGCGGGCCCTGGGGTCTCAGCG
WT	CGGCCCGACGAGCCATGGTTGCTGGGAGCGACGCGGGGCGGGCCCTGGGGTCTCAGCG

Allele-2: 2 bp deletion in exon 1.

Sanger Sequencing - Human CD58 knockout HeLa cell line (ab265947)

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