abcam

Product datasheet

Human ICAM1 knockout HeLa cell line ab261742

7 Images

Overview

Product name	Human ICAM1 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB, ICC
Biosafety level	2
General notes	**Recommended control:** Human wild-type HeLa cell line (ab255448). 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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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
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	ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin alpha-L/beta-2). During leukocyte trans-endothelial migration, ICAM1 engagement promotes the assembly of endothelial apical cups through ARHGEF26/SGEF and RHOG activation. In case of rhinovirus infection acts as a cellular receptor for the virus.
Sequence similarities	Belongs to the immunoglobulin superfamily. ICAM family. Contains 5 Ig-like C2-type (immunoglobulin-like) domains.
Post-translational modifications	Monoubiquitinated, which is promoted by MARCH9 and leads to endocytosis.
Cellular localization	Membrane.

Applications

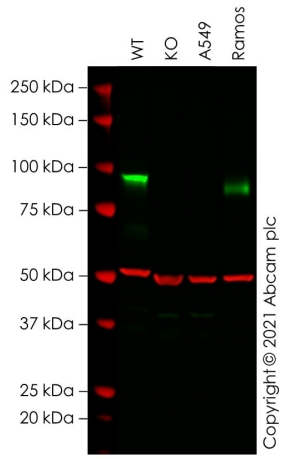
The Abpromise guarantee Our Abpromise guarantee covers the use of ab261742 in the following tested applications.

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: 57 kDa.

Application	Abreviews	Notes
ICC		Use at an assay dependent concentration.

Images



Western blot - Human ICAM1 knockout HeLa cell line (ab261742)

All lanes : Anti-ICAM1 antibody [EP1442Y] (ab53013) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ICAM1 knockout HeLa cell lysate
Lane 3 : A549 cell lysate
Lane 4 : Ramos cell lysate

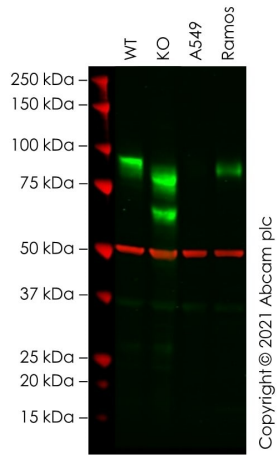
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 57 kDa
Observed band size: 90 kDa

False colour image of Western blot: Anti-ICAM1 antibody [EP1442Y] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab53013 was shown to bind specifically to ICAM1. A band was observed at 90 kDa in wild-type HeLa cell lysates with no signal observed at this size in Icam1 knockout cell line ab261742 (knockout cell lysate ab256947). The band observed in the knockout lysate lane below 90 kDa (not observed by this antibody) is likely to represent a truncated form of ICAM1. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and Icam1 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 ICAM1 knockout HeLa cell line (ab261742)

All lanes : Anti-ICAM1 antibody [EPR4776] (ab109361) at 1/1000 dilution

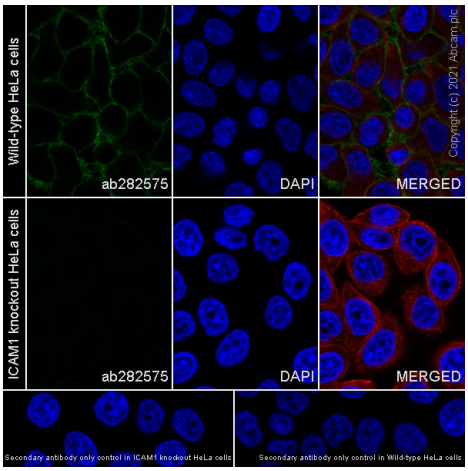
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ICAM1 knockout HeLa cell lysate
Lane 3 : A549 cell lysate
Lane 4 : Ramos cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 57 kDa
Observed band size: 90 kDa

False colour image of Western blot: Anti-ICAM1 antibody [EPR4776] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab109361 was shown to bind specifically to ICAM1. A band was observed at 90 kDa in wild-type HeLa cell lysates with no signal observed at this size in Icam1 knockout cell line ab261742 (knockout cell lysate ab256947). The band observed in the knockout lysate lane below 90 kDa is likely to represent a truncated form of ICAM1. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and Icam1 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.

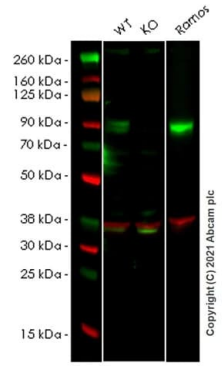


Immunocytochemistry - Human ICAM1 knockout HeLa cell line (ab261742)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized ICAM1 KO HeLa cells (ab261742) labelling ICAM1 with ab282575 at 1/500 (1.082 µg/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in wild-type HeLa cells, and no staining in ICAM1 knockout HeLa cells is observed.

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



Western blot - Human ICAM1 knockout HeLa cell line (ab261742)

All lanes :

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : ICAM1 knockout HeLa whole cell lysate
Lane 3 : Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysate

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (ab216776) at 1/10000 dilution

Predicted band size: 57 kDa

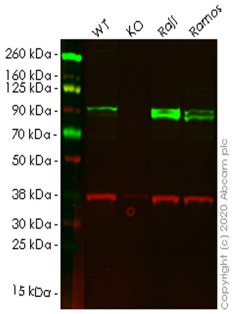
Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lanes 1-3: Merged signal (red and green). Green - ab282575 observed at 90kDa. Red - loading control ab8245 observed at 36 kDa.

ab282575 Anti-ICAM1 antibody [EPR24639-3] was shown to react with ICAM1 in wild-type Hela cells in Western blot. Loss of signal was observed when knockout cell line ab261742 (knockout cell lysate ab256947) was used. Wild-type and ICAM1 knockout

samples were subjected to SDS-PAGE.

ab282575 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at 4°C overnight 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 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Human ICAM1 knockout HeLa cell line (ab261742)

All lanes : Anti-ICAM1 antibody [EP1442Y] (ab53013) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ICAM1 knockout HeLa cell lysate
Lane 3 : Raji cell lysate
Lane 4 : Ramos 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: 57 kDa

Lanes 1-4: Merged signal (red and green). Green - ab53013 observed at 90 kDa. Red - loading control ab8245 observed at 36 kDa.

ab53013 Anti-ICAM1 antibody [EP1442Y] was shown to specifically react with ICAM1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261742 (knockout cell lysate ab256947) was used. Wild-type and ICAM1 knockout samples were subjected to SDS-PAGE. ab53013 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.

Sanger Sequencing - Human ICAM1 knockout HeLa cell line (ab261742)

Allele-1: Insertion of the selection cassette in exon 2.

Sanger Sequencing - Human ICAM1 knockout HeLa cell line (ab261742)

Allele-2: 1 bp insertion in exon 2.

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