abcam

Product datasheet

Human ICAM1 knockout HeLa cell line ab261742

画像数 7

製品の概要

製品名	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)
アプリケーション	**適用あり:** WB, ICC
Biosafety level	2
特記事項	**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.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
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
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	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.
配列類似性	Belongs to the immunoglobulin superfamily. ICAM family. Contains 5 Ig-like C2-type (immunoglobulin-like) domains.
翻訳後修飾	Monoubiquitinated, which is promoted by MARCH9 and leads to endocytosis.
細胞内局在	Membrane.

アプリケーション

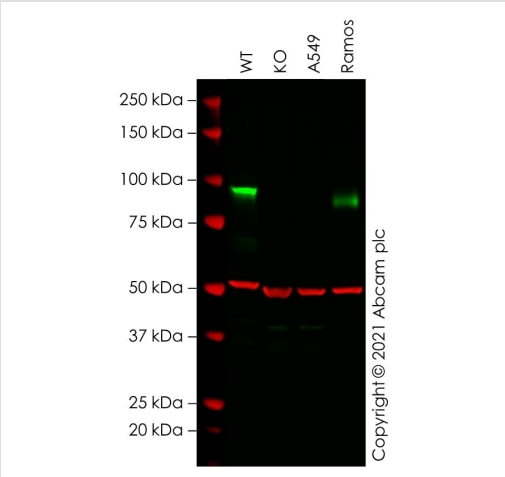
The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab261742の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 57 kDa.

アプリケーション	Abreviews	特記事項
ICC		Use at an assay dependent concentration.

画像



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

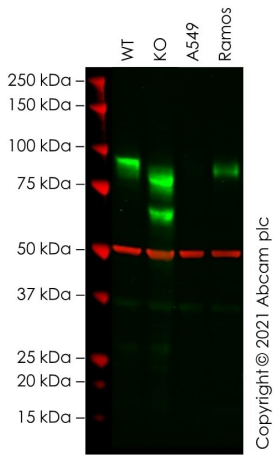
Lysates/proteins at 20 µg per lane.

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

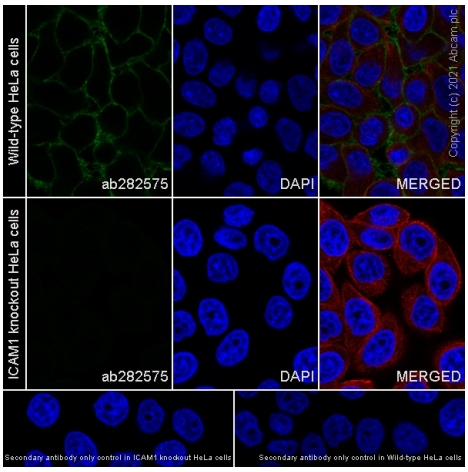
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ICAM1 knockout HeLa cell lysate
Lane 3 : A549 cell lysate
Lane 4 : Ramos cell lysate

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.

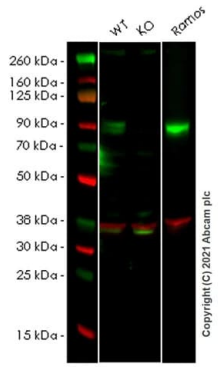


Immunocytochemistry - Human ICAM1 knockout HeLa cell line (ab261742)

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

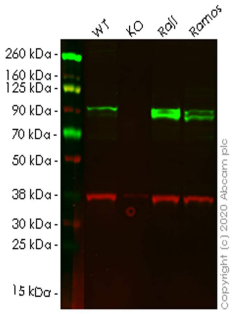
Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

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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