abcam

Product datasheet

Human ICAM1 knockout HeLa cell lysate ab256947

6 Images

Overview

Product name	Human ICAM1 knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
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	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. 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**.
Tested applications	**Suitable for:** WB

Properties

Storage instructions	Store at -80°C. Please refer to protocols.

Components	1 kit
ab261925 - Human ICAM1 knockout HeLa cell lysate	1 x 100µg
ab255552 - Human wild-type HeLa cell lysate	1 x 100µg

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

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.

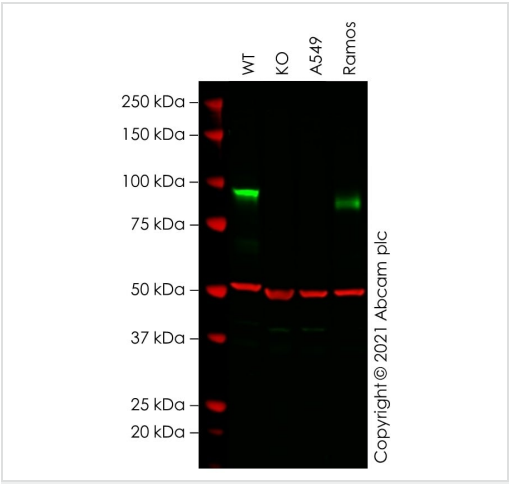
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256947 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.

Images



Western blot - Human ICAM1 knockout HeLa cell lysate (ab256947)

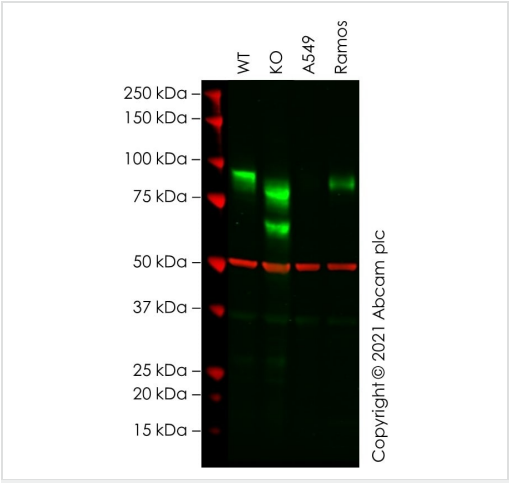
Lane 1: Wild-type HeLa cell lysate 20 µg

Lane 2: ICAM1 knockout HeLa cell lysate 20 µg

Lane 3: A549 cell lysate 20 µg

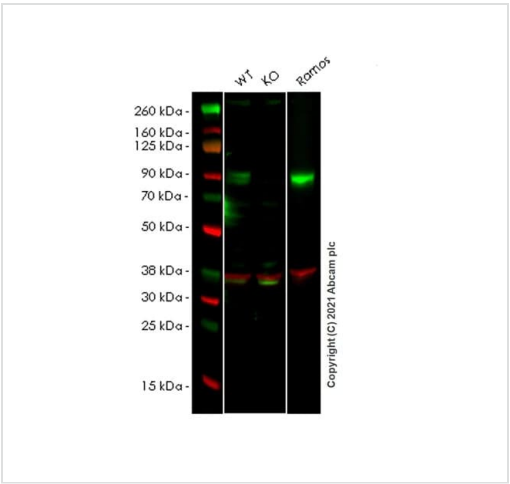
Lane 4: Ramos cell lysate 20 µg

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 (not observed by this antibody) 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.



Western blot - Human ICAM1 knockout HeLa cell lysate (ab256947)

Lane 1: Wild-type HeLa cell lysate 20 µg
Lane 2: ICAM1 knockout HeLa cell lysate 20 µg
Lane 3: A549 cell lysate 20 µg
Lane 4: Ramos cell lysate 20 µg
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.



Western blot - Human ICAM1 knockout HeLa cell lysate

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

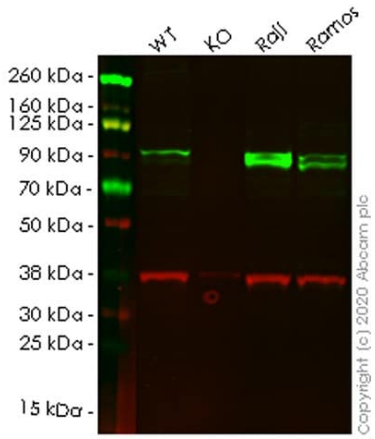
Lane 1: Wild-type HeLa cell lysate
Lane 2: ICAM1 knockout HeLa cell lysate
Lane 3: Ramos cell lysate

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 lysate (ab256947)

Lane 1: Wild-type HeLa cell lysate (20 ug)
Lane 2: ICAM1 knockout HeLa cell lysate (20 ug)
Lane 3: Raji cell lysate (20 ug)
Lane 4: Ramos cell lysate (20 ug)

ab53013 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 lysate (ab256947)

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

Sanger Sequencing - Human ICAM1 knockout HeLa cell lysate (ab256947)

Allele-2: 1 bp insertion in exon 2

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