abcam

Product datasheet

Human VCAM1 knockout A549 cell line ab273758

4 Images

Overview

Product name	Human VCAM1 knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 4 bp deletion in exon 2
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB, ICC
Biosafety level	1
General notes	**Recommended control:** Human wild-type A549 cell line (**ab275463**). 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:** F-12K + 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 $2x10^3$-$1x10^4$ 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 $6x10^4$ 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.
Do not exceed $7x10^4$ cells/cm^2.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

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	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
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	Important in cell-cell recognition. Appears to function in leukocyte-endothelial cell adhesion. Interacts with the beta-1 integrin VLA4 on leukocytes, and mediates both adhesion and signal transduction. The VCAM1/VLA4 interaction may play a pathophysiologic role both in immune responses and in leukocyte emigration to sites of inflammation.
Tissue specificity	Expressed on inflammed vascular endothelium, as well as on macrophage-like and dendritic cell types in both normal and inflammed tissue.
Sequence similarities	Contains 7 Ig-like C2-type (immunoglobulin-like) domains.
Domain	Either the first or the fourth Ig-like C2-type domain is required for VLA4-dependent cell adhesion.
Post-translational modifications	Sialoglycoprotein.
Cellular localization	Membrane.

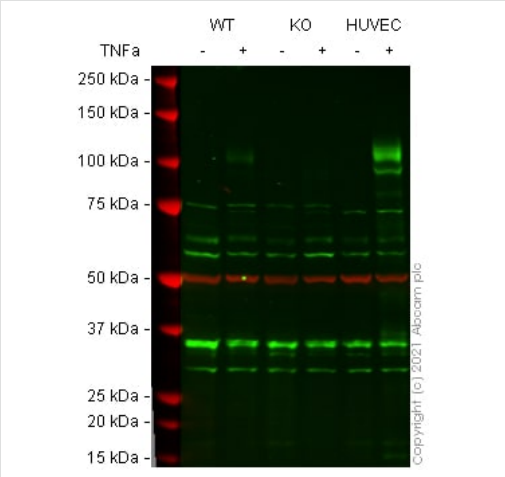
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab273758 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.
ICC		Use at an assay dependent concentration.

Images



Western blot - Human VCAM1 knockout A549 cell line (ab273758)

All lanes : Anti-VCAM1 antibody [EPR5047] (**ab134047**) at 1/2000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : Wild-type A549 TNF-a treated (10 ng/mL, 16h) cell lysate
Lane 3 : VCAM1 knockout A549 cell lysate
Lane 4 : VCAM1 knockout A549 TNF-a treated (10 ng/mL, 16h) cell lysate
Lane 5 : HUVEC cell lysate
Lane 6 : HUVEC TNF-a treated (16 ng/mL, 16h) cell lysate

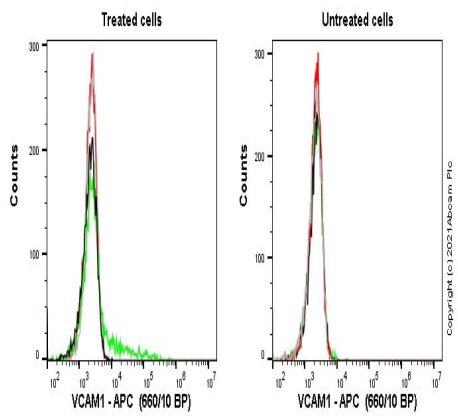
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 105 kDa

Lanes 1 - 6: Merged signal (red and green). Green - **ab134047** observed at 105 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab134047 was shown to react with VCAM1 in treated wild-type A549 cells in Western blot with loss of signal observed in treated VCAM1 knockout cell line ab273758 (knockout cell lysate **ab275504**). Wild-type A549 and VCAM1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab134047** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

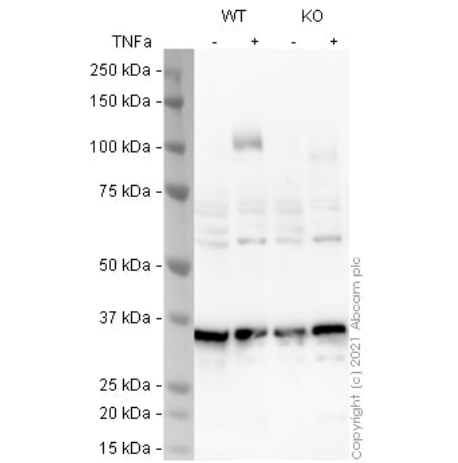


Flow Cytometry - Human VCAM1 knockout A549 cell line (ab273758)

Flow cytometry overlay histogram showing wild-type A549 (green line) and VCAM1 knockout A549 cells (red line, ab273758), treated with 10 ng/ml TNF-alpha for 16 h (left) and untreated (right), stained with **ab103173**. The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab103173**) ($1x10^6$ in 100µl at 0.2µg/ml) for 30 min at 4°C.

Isotype control antibody mouse IgG1κ Allophycocyanin was used at the same concentration and conditions as the primary antibody (wild-type A549 - black line VCAM knockout A549 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 40 mW Red laser (638nm) and 660/10 bandpass filter.



Western blot - Human VCAM1 knockout A549 cell line (ab273758)

All lanes : Anti-VCAM1 antibody [EPR5038(2)] (**ab174279**) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : Wild-type A549 TNF-a treated (10 ng/mL, 16h) cell lysate
Lane 3 : VCAM1 knockout A549 cell lysate
Lane 4 : VCAM1 knockout A549 TNF-a treated (10 ng/mL, 16h) cell lysate

Lysates/proteins at 30 µg per lane.

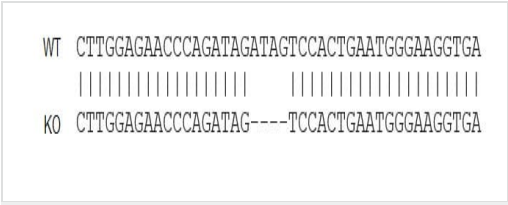
Performed under reducing conditions.

Observed band size: 105 kDa

Exposure time: 20 seconds

ab174279 was shown to react with VCAM1 in treated wild-type A549 cells in western blot. Loss of signal was observed when treated VCAM1 knockout cell line ab273758 (knockout cell lysate **ab275504**) was used. Membranes were blocked in 3 % milk in

TBS-T (0.1 % Tween®) before incubation with **ab174279** overnight at 4 °C at a 1 in 1000 dilution. Blots were incubated with HRP conjugated Goat anti-Rabbit (H+L) secondary antibody at 1 in 5000 for 1 hour at room temperature before development with Optiblot ECL reagent (**ab133456**) and imaging.



Sanger Sequencing - Human VCAM1 knockout A549 cell line (ab273758)

Allele-1: 4 bp deletion in exon 2

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