

### Human VCAM1 knockout A549 cell line ab273758

4 Images

#### Overview

<b>Product name</b>	Human VCAM1 knockout A549 cell line
<b>Parental Cell Line</b>	A549
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, Homozygous: 4 bp deletion in exon 2
<b>Passage number</b>	<20
<b>Knockout validation</b>	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
<b>Tested applications</b>	<b>Suitable for:</b> WB, ICC
<b>Biosafety level</b>	1
<b>General notes</b>	<p><b>Recommended control:</b> Human wild-type A549 cell line (<a href="#">ab275463</a>). 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><b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p><b>Culture medium:</b> F-12K + 10% FBS</p> <p><b>Initial handling guidelines:</b> 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"> <li>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>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.</li> <li>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 <math>2 \times 10^3</math>-<math>1 \times 10^4</math> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> <p><b>Subculture guidelines:</b></p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of <math>6 \times 10^4</math> cells/cm<sup>2</sup> 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.

Do not exceed  $7 \times 10^4$  cells/cm<sup>2</sup>.

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We will provide viable cells that proliferate on revival.

## Properties

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<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Lung
<b>Cell type</b>	epithelial
<b>Disease</b>	Carcinoma
<b>Gender</b>	Male
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

## Target

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<b>Function</b>	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.
<b>Tissue specificity</b>	Expressed on inflamed vascular endothelium, as well as on macrophage-like and dendritic cell types in both normal and inflamed tissue.
<b>Sequence similarities</b>	Contains 7 Ig-like C2-type (immunoglobulin-like) domains.
<b>Domain</b>	Either the first or the fourth Ig-like C2-type domain is required for VLA4-dependent cell adhesion.
<b>Post-translational modifications</b>	Sialoglycoprotein.
<b>Cellular localization</b>	Membrane.

## Applications

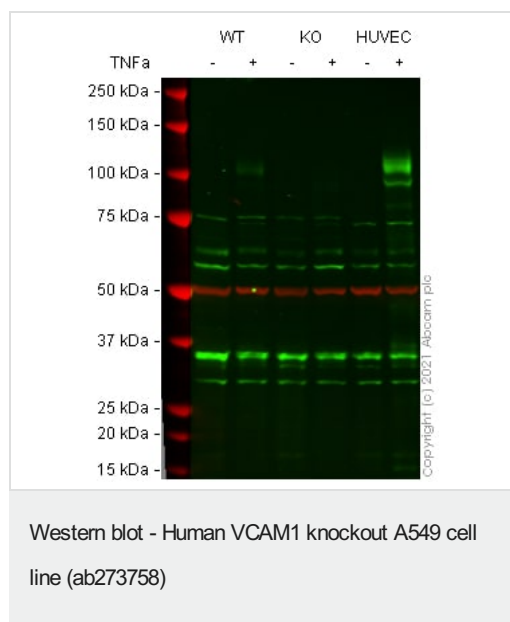
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**The Abpromise guarantee** Our [Abpromise guarantee](#) covers the use of ab273758 in the following tested applications.

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



**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

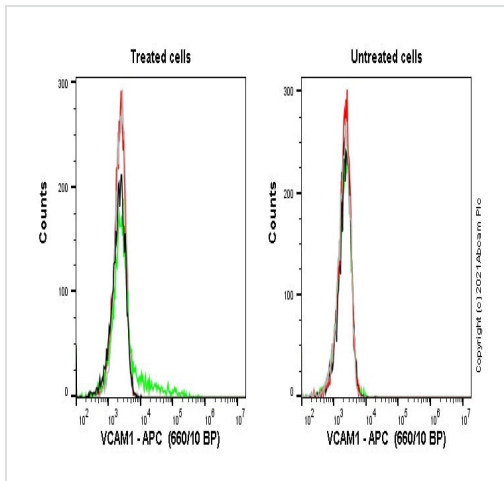
Lysates/proteins at 30 µg per lane.

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<sup>®</sup>) 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<sup>®</sup> 680RD) preabsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

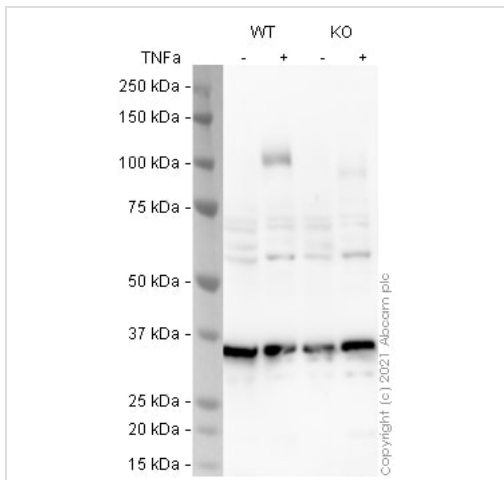


Flow Cytometry - Human VCAM1 knockout A549 cell line (ab273758)

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**) ( $1 \times 10^6$  in 100  $\mu$ l at 0.2  $\mu$ g/ml) for 30 min at 4°C.

Isotype control antibody mouse IgG1 $\kappa$  Allophycocyanin was used at the same concentration and conditions as the primary antibody (wild-type A549 - black line VCAM1 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  $\mu$ g per lane.

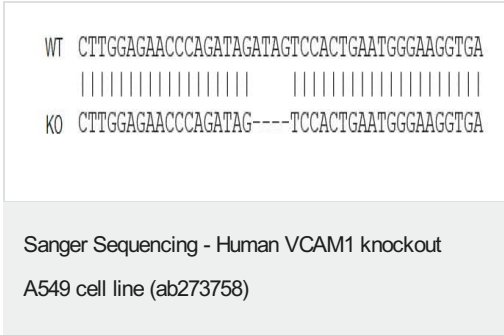
Performed under reducing conditions.

**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.



Allele-1: 4 bp deletion in exon 2

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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