

Human VCAM1 knockout A549 cell line ab273758

4 图像

概述

产品名称	人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)
经测试应用	适用于: WB, ICC
Biosafety level	1
常规说明	<p>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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: F-12K + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^3-1×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 6×10^4 cells/cm² 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 7×10^4 cells/cm².

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We will provide viable cells that proliferate on revival.

性能

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
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

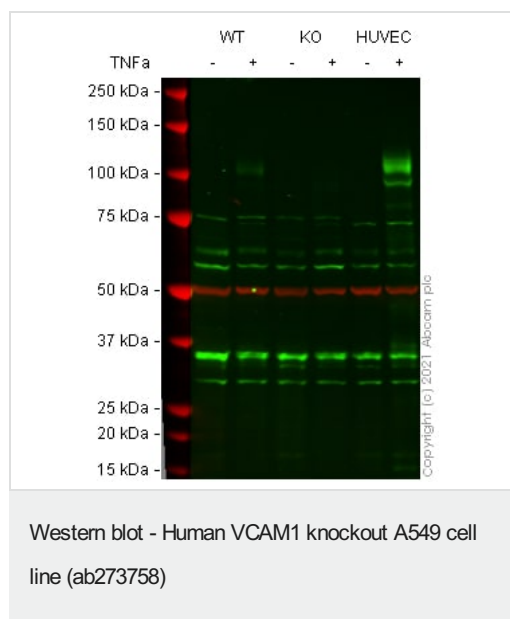
功能	Important in cell-cell recognition. Appears to function in leukocyte-endothelial cell adhesion. Interacts with the beta-1 integrin VLA4 on leukocytes, and mediates both adhesion and signal transduction. The VCAM1/VLA4 interaction may play a pathophysiologic role both in immune responses and in leukocyte emigration to sites of inflammation.
组织特异性	Expressed on inflamed vascular endothelium, as well as on macrophage-like and dendritic cell types in both normal and inflamed tissue.
序列相似性	Contains 7 Ig-like C2-type (immunoglobulin-like) domains.
结构域	Either the first or the fourth Ig-like C2-type domain is required for VLA4-dependent cell adhesion.
翻译后修饰	Sialoglycoprotein.
细胞定位	Membrane.

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab273758于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.



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

Lane 1 : Wild-type A549 cell lysate

Lane 2 : Wild-type A549 TNF-α treated (10 ng/mL, 16h) cell lysate

Lane 3 : VCAM1 knockout A549 cell lysate

Lane 4 : VCAM1 knockout A549 TNF-α treated (10 ng/mL, 16h) cell lysate

Lane 5 : HUVEC cell lysate

Lane 6 : HUVEC TNF-α treated (16 ng/mL, 16h) cell lysate

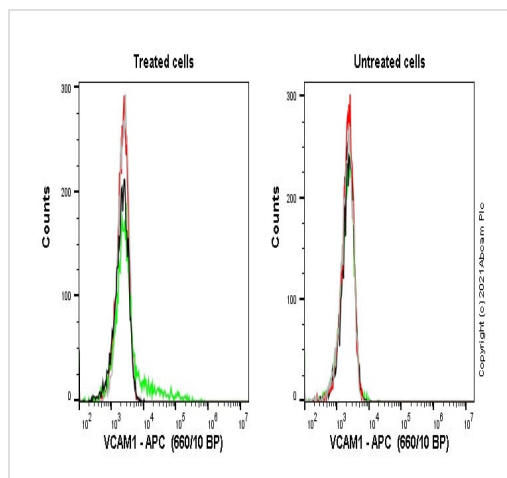
Lysates/proteins at 30 µg per lane.

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.



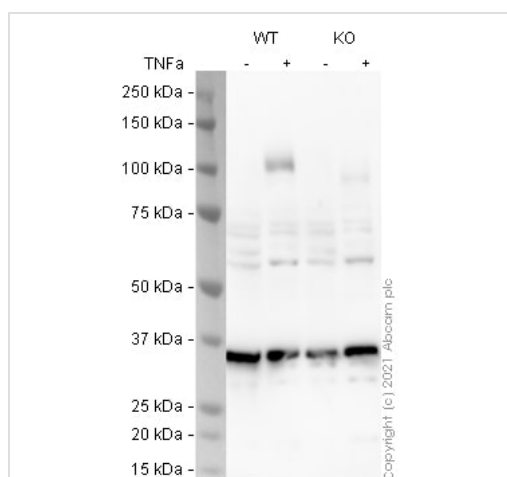
Flow Cytometry - Human VCAM1 knockout A549 cell line (ab273758)

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**) (1×10^6 in 100 μ l at 0.2 μ g/ml) for 30 min at 4°C.

Isotype control antibody mouse IgG1 κ 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 μ 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.

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WT  CTTGGAGAACCAGATAGTAGTCCACTGAATGGGAAGGTGA
    |||||
KO  CTTGGAGAACCAGATAG---TCCACTGAATGGGAAGGTGA
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Sanger Sequencing - Human VCAM1 knockout
A549 cell line (ab273758)

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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