abcam

Product datasheet

Human VCAM1 knockout A549 cell lysate ab275504

3 图像

概述

产品名称

人VCAM1 knockout A549 cell裂解物

产品概述

Knockout cell lysate achieved by CRISPR/Cas9.

Treatments:

Human VCAM1 knockout A549 cell lysate - Untreated

Human wild-type A549 cell lysate - Untreated

Human VCAM1 knockout A549 cell lysate - TNF-a (10 ng/ml, 16h)

Human wild-type A549 cell lysate - TNF-a (10 ng/ml, 16h)

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 Sanger Sequencing, Western Blot (WB)

 $\label{eq:Reconstitution notes} \textbf{To use as WB control, resuspend the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the lyophilizate in 50 μL of LDS* Sample Buffer to have a final labeled and the labele$

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.

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.

Authorisation, and any other relevant authorisations, for their intended uses.

It is the responsibility of our customers to check the necessity of application of REACH

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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 <u>limited use license</u> and <u>patent pages</u>.

经测试应用 适用于: WB

性能

存放说明 Store at -80°C. Please refer to protocols.

组 件	1 kit
ab277498 - Human VCAM1 knockout A549 cell lysate - TNF-alpha treated	1 x 100µg
ab277312 - Human VCAM1 knockout A549 cell lysate	1 x 100µg
ab277499 - Human wild-type A549 cell lysate - TNF-alpha treated	1 x 100µg
ab277305 - Human wild-type A549 cell lysate	1 x 100µg

Cell typeepithelialDiseaseCarcinomaGenderMale

靶标

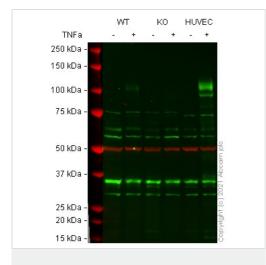
功能	Important in cell-cell recognition. Appears to function in leukocyte-endothelial cell adhesion. Interacts with the beta-1 integrin VLA4 on leukocytes, and mediates both adhesion and signal transduction. The VCAM1/VLA4 interaction may play a pathophysiologic role both in immune responses and in leukocyte emigration to sites of inflammation.
组织 特异性	Expressed on inflammed vascular endothelium, as well as on macrophage-like and dendritic cell types in both normal and inflammed tissue.
序列相似性	Contains 7 lg-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

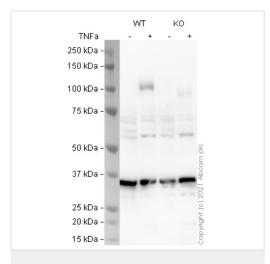
The Abpromise guarantee Abpromise™承诺保证使用ab275504于以下的经测试应用

"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.



Western blot - Human VCAM1 knockout A549 cell lysate (ab275504)



Western blot - Human VCAM1 knockout A549 cell lysate (ab275504)

Lane 1: Wild-type A549 cell lysate 30 ug

Lane 2: Wild-type A549 TNF-a treated (10 ng/mL, 16h) cell lysate 30 ug

Lane 3: VCAM1 knockout A549 cell lysate 30 ug

Lane 4: VCAM1 knockout A549 TNF-a treated (10 ng/mL, 16h) cell lysate 30 ug

Lane 5: HUVEC cell lysate 30 ug

Lane 6: HUVEC TNF-a treated (16 ng/mL, 16h) cell lysate 30 ug

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

ab134047 was shown to react with VCAM1 in wild-type A549 cells in Western blot with loss of signal observed in 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.

Lane 1: Wild-type A549 cell lysate 30 ug

Lane 2: Wild-type A549 TNF-a treated (10 ng/mL, 16h) cell lysate 30 ug

Lane 3: VCAM1 knockout A549 cell lysate 30 ug

Lane 4: VCAM1 knockout A549 TNF-a treated (10 ng/mL, 16h) cell lysate 30 ug

Lanes 1: Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug

Lanes 2: GPX4 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug

Lanes 3: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate, 20 ug

Lanes 3: Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate, 20 ug

<u>ab174279</u> 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 <u>ab273758</u> (knockout cell lysate ab275504) was used. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween[®]) before incubation with <u>ab174279</u> 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.

WT CTTGGAGAACCCAGATAGATAGTCCACTGAATGGGAAGGTGA KO CTTGGAGAACCCAGATAG----TCCACTGAATGGGAAGGTGA

Sanger Sequencing - Human VCAM1 knockout A549 cell lysate (ab275504)

Allele-1: 4 bp deletion in exon 2

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