abcam

Product datasheet

Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free ab215380

KO VALIDATED Recombinant RabMAb

9 References 10 Images

Overview

Product name	Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR5047] to VCAM1 - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), Indirect ELISA, WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human fetal liver, HuT 78, NIH 3T3, Mouse brain, Mouse kidney, Mouse spleen, Rat brain, Rat kidney and Rat spleen lysates; Wild-type HUVEC and A549 TNF-a treated (10 ng/mL, 16h) cell lysates. IHC-P: Human and Mouse spleen FFPE tissue sections. Flow Cyt (intra): K562 cells. ICC/IF: HUVEC TNF-a treated (10 ng/mL, 16h); K562 cells.
General notes	ab215380 is the carrier-free version of **ab134047**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5047
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab215380 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
Indirect ELISA		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		Use at an assay dependent concentration.

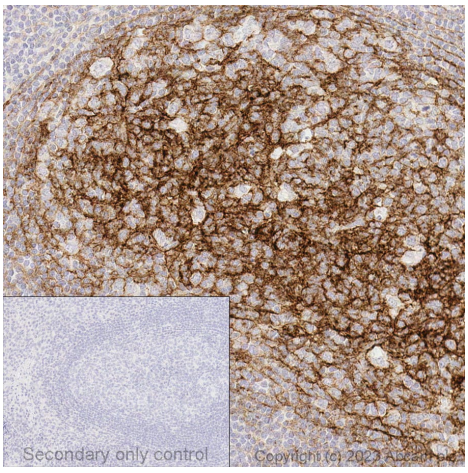
Target

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.

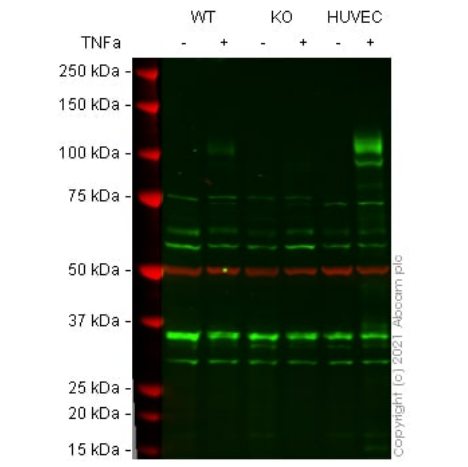
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

Immunohistochemical analysis of formalin-fixed paraffin-embedded human tonsil labelling VCAM1 with **ab271899** at a concentration of 0.5µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). **ab271899** anti VCAM1 antibody was incubated at 37°C for 16min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

This data was developed using the same antibody clone in a different buffer formulation (**ab271899**).



Western blot - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

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.

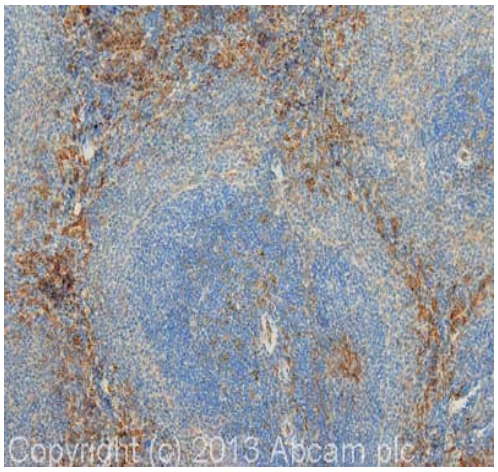
Performed under reducing conditions.

Observed band size: 105 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab134047**).

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 wild-type A549 cells in Western blot with loss of signal observed in VCAM1 knockout sample. 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.

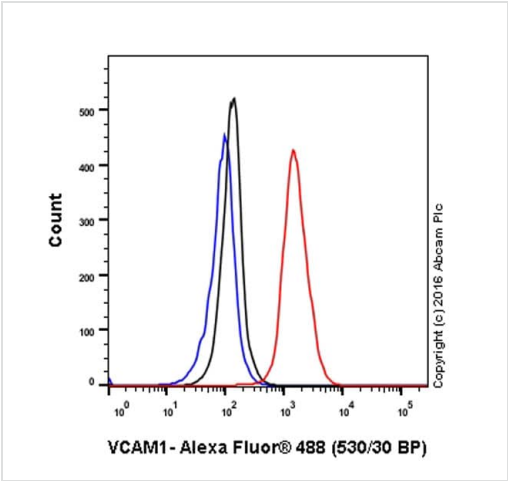


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

IHC image of VCAM1 staining in Mouse spleen formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with unpurified **ab134047**, 1/200 dilution, for 15 mins at room temperature. A goat anti-rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

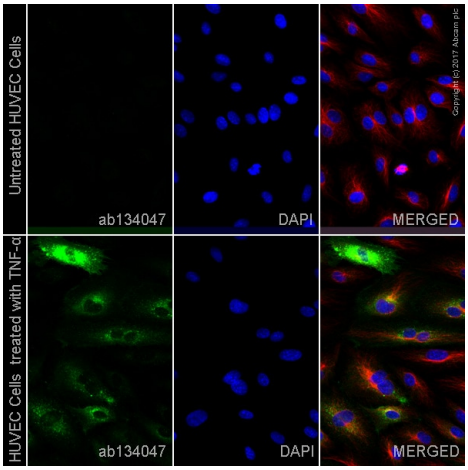
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134047**).



Flow Cytometry (Intracellular) - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

Intracellular Flow Cytometry analysis of K562 (human chronic myelogenous leukemia) cells labeling VCAM1 with purified **ab134047** at 1/40 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor®488) at 1/2000 dilution was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.

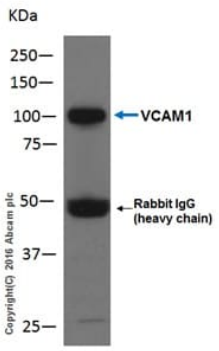
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134047**).



Immunocytochemistry/ Immunofluorescence - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

ab134047 staining VCAM1 in HUVEC cells treated with TNF-α (**ab55237**) at 10 ng/ml for 16 hours. The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab134047** at 2µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 647) (**ab150119**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134047**).



Immunoprecipitation - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

VCAM1 was immunoprecipitated from Human fetal liver lysate with **ab134047** at 1/110 dilution.

Western blot was performed from the immunoprecipitate using **ab134047** at 1/1000 dilution.

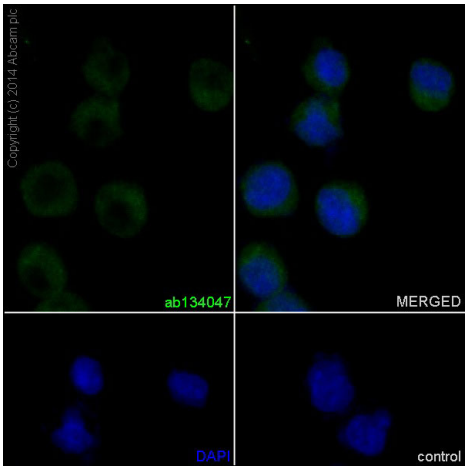
VeriBlot for IP secondary antibody (Peroxidase conjugated),was used as secondary antibody at 1/1000 dilution.

Lane 1:Human fetal liver lysate

Blocking and dilution buffer:5% NFDM/TBST

Exposure time: 1 second

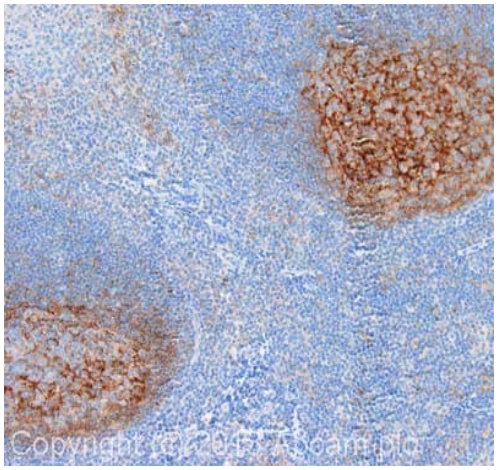
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134047**).



Immunocytochemistry/ Immunofluorescence - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

Immunofluorescent staining of K562 cells (fixed in 4% PFA, permeabilized with 0.1% Triton X 100) using purified **ab134047** at a dilution of 1/250. An Alexa Fluor® 488 goat anti-rabbit antibody was used as the secondary at a dilution of 1/500 and the cells were counter stained with DAPI. The negative control is shown in the bottom right hand panel - for the negative control, Alex Fluor® 594 goat anti-mouse was used at a dilution of 1/500.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134047**).

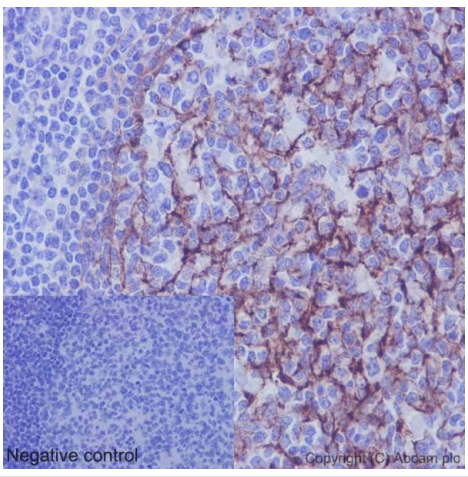


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

This IHC data was generated using the same anti-VCAM1 antibody clone, EPR5047, in a different buffer formulation (cat# **ab134047**).

IHC image of VCAM1 staining in Human spleen formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with unpurified **ab134047**, 1/200 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

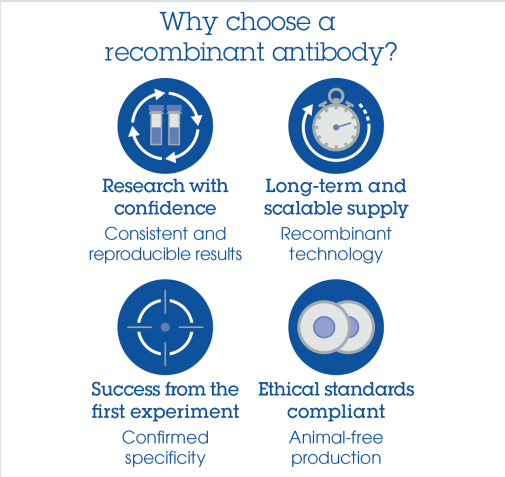
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

This IHC data was generated using the same anti-VCAM1 antibody clone, EPR5047, in a different buffer formulation (cat# **ab134047**).

Immunohistochemical staining of paraffin embedded human tonsil with purified **ab134047** at a dilution of 1/500. A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

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