abcam

Product datasheet

Anti-VCAM1 antibody [EPR5047] ab134047

KO VALIDATED Recombinant RabMAb

16 Abreviews 258 References 19 Images

Overview

Product name	Anti-VCAM1 antibody [EPR5047]
Description	Rabbit monoclonal [EPR5047] to VCAM1
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, IHC-P, Flow Cyt (Intra), ICC/IF, Indirect ELISA
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human spleen and tonsil tissue; mouse spleen tissue. WB: Mouse kidney, brain and spleen tissue lysate; rat brain, spleen and kidney tissue lysate; human fetal liver tissue lysate; NIH/3T3, LADMAC, HuT-78, TNF-a treated HUVEC, and LPS treated bEnd.3 cell lysates; Wild-type A549 and HUVEC TNF-a treated (10 ng/mL, 16h) cell lysate Flow Cyt (intra): K562 cells. ICC/IF: HUVEC TNF-a treated (10 ng/mL, 16h); K562 cells. IP: Human fetal liver tissue lysate.
General notes	Vascular cell adhesion protein 1 (VCAM) is a protein that is encoded by the VCAM1 gene in humans. It plays a role in functioning as a cell adhesion molecule and is thought to participate in monocyte recruitment to atherosclerotic sites, and as such is highly overexpressed in brain inflammation. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide

	Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5047
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab134047 in the following tested applications.

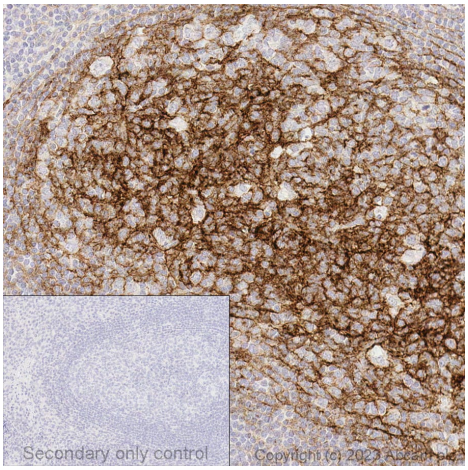
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (3)	1/2000 - 1/10000. Detects a band of approximately 100 kDa (predicted molecular weight: 81 kDa). Stimulation may be required to allow detection of the target protein due to low levels of endogenous expression in some samples. Please see images below for recommended treatment conditions and positive controls.
IP		1/40.
IHC-P	★★★★★ (5)	1/500 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Flow Cyt (Intra)		1/40.
ICC/IF	★★★★★ (3)	1/250.
Indirect ELISA		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.

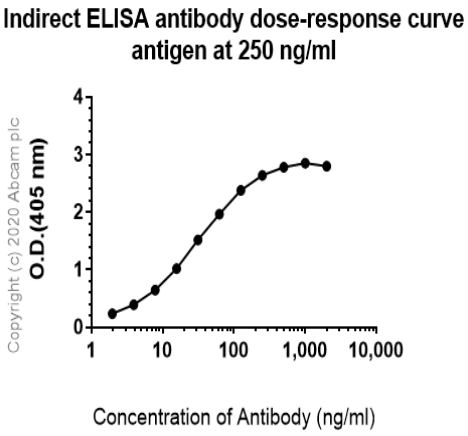
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] (ab134047)

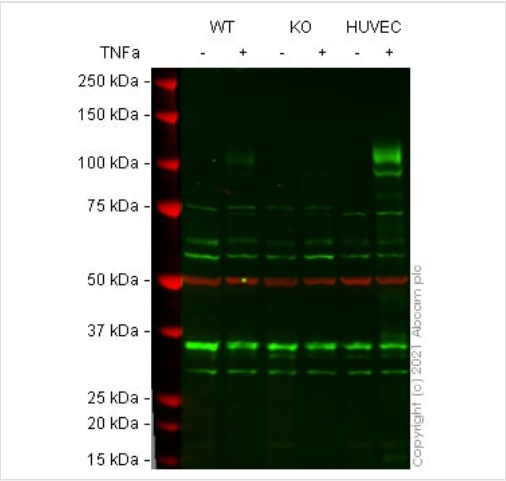
Immunohistochemical analysis of formalin-fixed paraffin-embedded human tonsil labelling VCAM1 with **ab271899** at a concentration of 0.5µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen 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**).



Indirect ELISA - Anti-VCAM1 antibody [EPR5047] (ab134047)

ELISA using ab134047 at varying antibody concentrations and antigen concentration at 250 ng/mL. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (1/2500) was used as the secondary antibody.



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

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

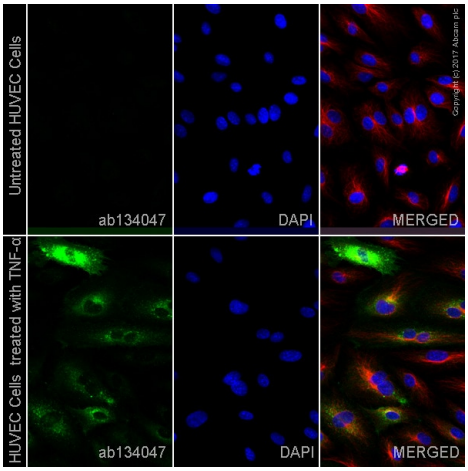
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 81 kDa
Observed band size: 105 kDa

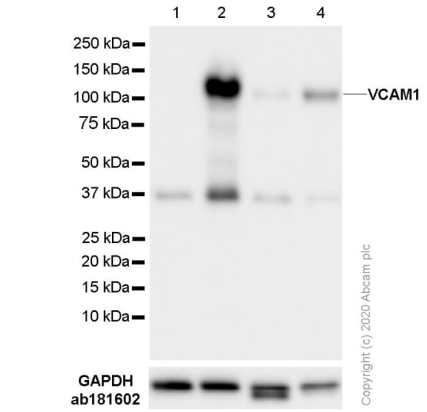
Lanes 1 - 6: Merged signal (red and green). Green - ab134047 observed at 105 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

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.



Immunocytochemistry/ Immunofluorescence - Anti-VCAM1 antibody [EPR5047] (ab134047)

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).



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

All lanes : Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/1000 dilution

Lane 1 : HUVEC (Human umbilical vein endothelial cell) whole cell lysate
Lane 2 : HUVEC (Human umbilical vein endothelial cell) treated with 10 ng/ml TNF-a for 16 h, whole cell lysate
Lane 3 : bEnd.3 (Mouse brain endothelioma) whole cell lysate
Lane 4 : bEnd.3 (Mouse brain endothelioma) treated with 10 µg/ml LPS for 24 h, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

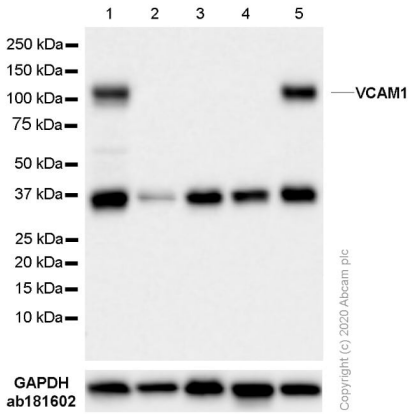
Predicted band size: 81 kDa
Observed band size: 100 kDa
Additional bands at: 36 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 seconds

Rabbit monoclonal [EPR16891] to GAPDH (**ab181602**) used as loading control.

Blocking/Diluting buffer: 5% NFDM/TBST.

Stimulation may be required to allow detection of the target protein due to low levels of endogenous expression in some samples.



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

All lanes : Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/1000 dilution

Lane 1 : Hut-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysate
Lane 2 : SK-OV-3 (Human ovarian cancer epithelial cell) whole cell lysate
Lane 3 : RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 4 : J774A.1 (Mouse reticulum cell sarcoma monocyte macrophage) whole cell lysate
Lane 5 : LADMAC (Mouse bone marrow monocyte macrophage) whole cell lysate

Lysates/proteins at 20 µg per lane.

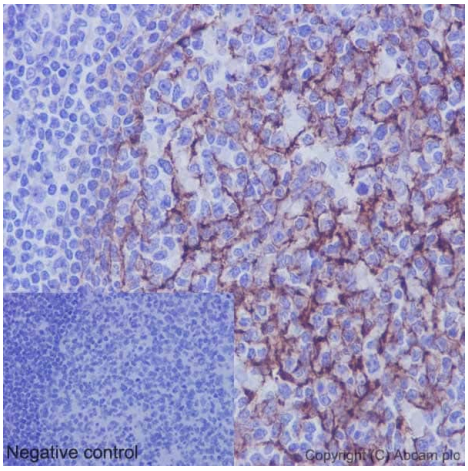
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 81 kDa
Observed band size: 100 kDa
Additional bands at: 36 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 7 seconds

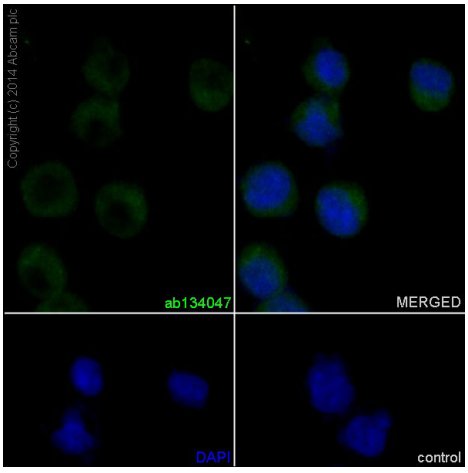
Rabbit monoclonal [EPR16891] to GAPDH (**ab181602**) used as loading control.

Blocking/Diluting buffer: 5% NFDM/TBST.



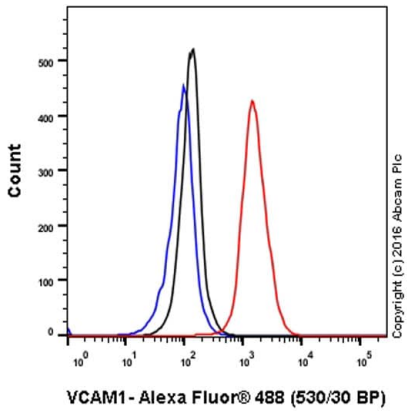
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] (ab134047)

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.



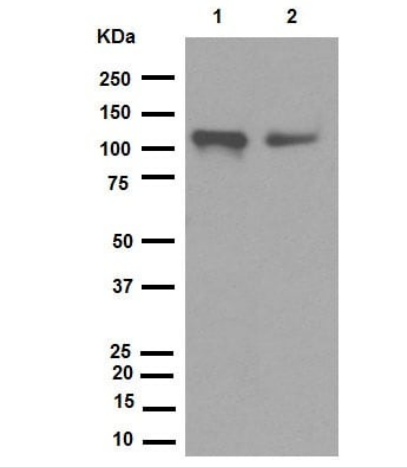
Immunocytochemistry/ Immunofluorescence - Anti-VCAM1 antibody [EPR5047] (ab134047)

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.



Flow Cytometry (Intracellular) - Anti-VCAM1 antibody [EPR5047] (ab134047)

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.



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

All lanes : Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/10000 dilution (purified)

Lane 1 : Mouse kidney
Lane 2 : Rat spleen

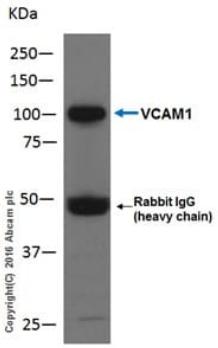
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 81 kDa
Observed band size: 100 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Immunoprecipitation - Anti-VCAM1 antibody [EPR5047] (ab134047)

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

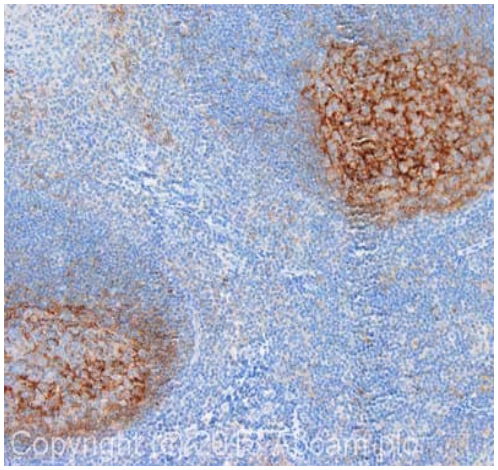
Western blot was performed from the immunoprecipitate using ab134047 at 1/1000 dilution.

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.

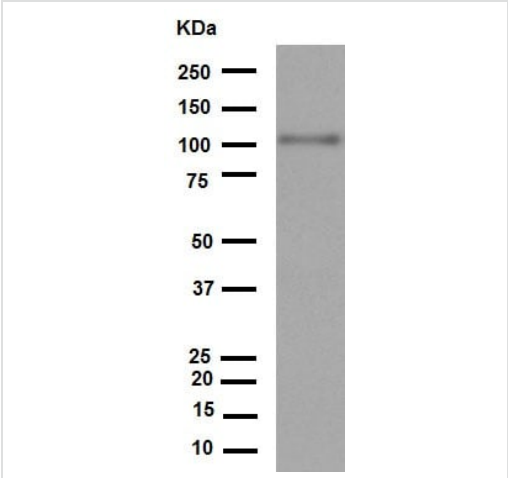
Exposure time: 1 second.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] (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.



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/2000 dilution (purified) + Rat kidney tissue lysate at 20 µg

Secondary

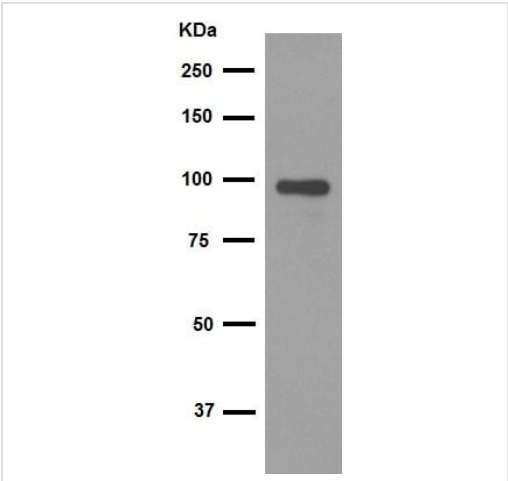
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 81 kDa

Observed band size: 100 kDa

Blocking buffer: 5% NFDM/TBST.

Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/10000 dilution (purified) + NIH/3T3 cell lysate at 10 µg

Secondary

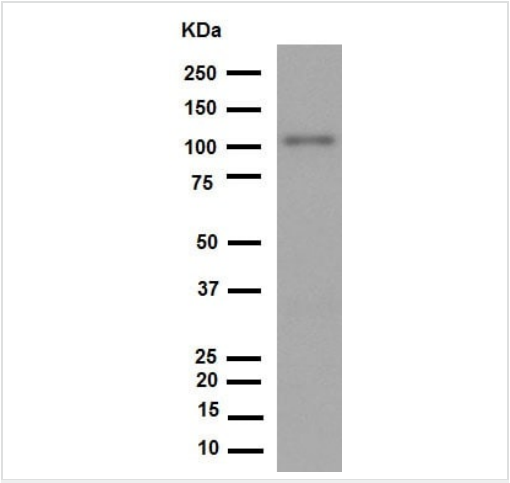
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 81 kDa

Observed band size: 100 kDa

Blocking buffer: 5% NFDM/TBST.

Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/10000 dilution (purified) + Human fetal liver tissue lysate at 20 µg

Secondary

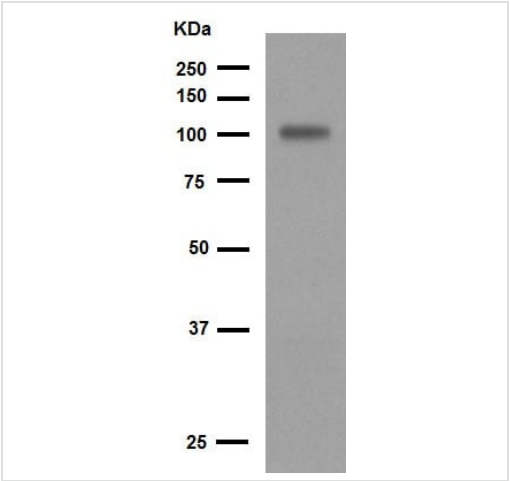
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 81 kDa

Observed band size: 100 kDa

Blocking buffer: 5% NFDM/TBST.

Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/10000 dilution (purified) + HuT-78 cell lysate at 10 µg

Secondary

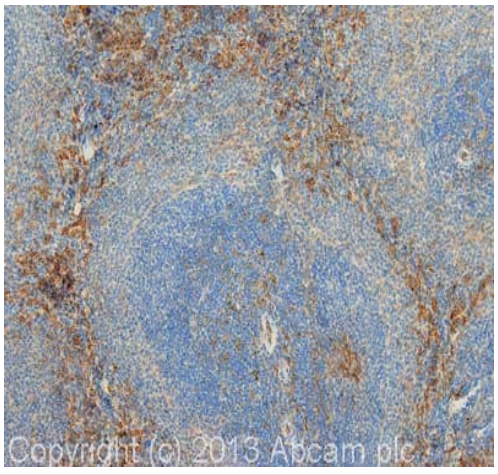
HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 81 kDa

Observed band size: 100 kDa

Blocking buffer: 5% NFDM/TBST.

Dilution buffer: 5% NFDM/TBST.

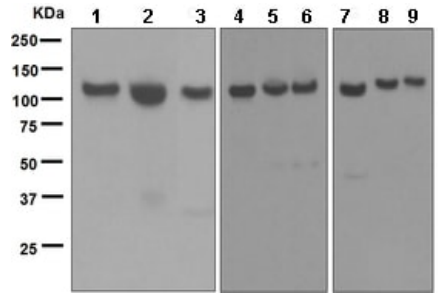


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] (ab134047)

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.



Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047)

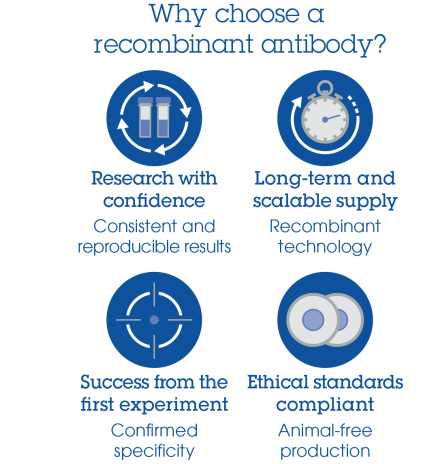
All lanes : Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/1000 dilution (unpurified)

Lane 1 : Human fetal liver tissue lysate
Lane 2 : HuT 78 cell lysate
Lane 3 : NIH/3T3 cell lysate
Lane 4 : Mouse brain tissue lysate
Lane 5 : Mouse kidney tissue lysate
Lane 6 : Mouse spleen tissue lysate
Lane 7 : Rat brain tissue lysate
Lane 8 : Rat kidney tissue lysate
Lane 9 : Rat spleen tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**) at 1/2000 dilution

Predicted band size: 81 kDa



Anti-VCAM1 antibody [EPR5047] (ab134047)

- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

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