abcam

ab215380

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

Recombinant | RabMAb | KO Validated

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Constituents: PBS |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Carrier free** | Yes |
| **Clone number** | EPR5047 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 1.014 - 1.031 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## I-ELISA

### Tested

| | |
|---|---|
| **Species** | Synthetic peptide |
| **Dilution info** | - |

| | |
|---|---|
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Human, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Mouse, Human |
| **Dilution info** | - |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide |
| **Dilution info** | - |
| **Notes** | - |

## IP

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | 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. |

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | - |
| **Notes** | 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. |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | - |
| **Notes** | 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. |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide |
| **Dilution info** | - |
| **Notes** | - |

# ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide |
| **Dilution info** | - |
| **Notes** | - |

# Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide |

| | |
|---|---|
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information VCAM1

| | |
|---|---|
| **Function** | Cell adhesion glycoprotein predominantly expressed on the surface of endothelial cells that plays an important role in immune surveillance and inflammation (PubMed:31310649). Acts as a major regulator of leukocyte adhesion to the endothelium through interaction with different types of integrins (PubMed:10209034). During inflammatory responses, binds ligands on the surface of activated endothelial cells to initiate the activation of calcium channels and the plasma membrane-associated small GTPase RAC1 leading to leukocyte transendothelial migration (PubMed:22970700). Also serves as a quality-control checkpoint for entry into bone marrow by providing a 'don't-eat-me' stamping in the context of major histocompatibility complex (MHC) class-I presentation (PubMed:35210567). |

## Storage

| | |
|---|---|
| **Shipped at conditions** | Blue Ice |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | +4°C |
| **Storage information** | Do Not Freeze |

## Notes

ab215380 is the carrier-free version of ab134047.

**Patented technology**

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**What are the advantages of a recombinant monoclonal antibody?**

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 batch production

For more information, read more on recombinant antibodies.

**Conjugation ready**
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

**Compatibility**
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.

**What does low endotoxin mean?**
Our low endotoxin, azide-free formats have low endotoxin level (1 EU/mg, determined by the TAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | VCAM1 also known as vascular cell adhesion molecule 1 plays an important role in mediating adhesion and signal transduction. VCAM1 has an approximate molecular mass of 110 kDa. It is expressed on the surface of endothelial cells and can be upregulated by cytokines released during inflammation. The protein serves as a ligand for the integrin VLA-4 also known as α4β1 and contributes to the adhesion of leukocytes to endothelial cells. |
| **Biological function summary** | Adhesion molecules like VCAM1 participate in immune response by recruiting leukocytes to sites of inflammation. VCAM1 helps guide immune cells to directed locations where they perform defense activities. Although VCAM1 is not part of any known large protein complex its interaction with integrins facilitates the migration of immune cells across the endothelium and into tissue. |
| **Pathways** | VCAM1 contributes significantly to the leukocyte extravasation process in inflammatory pathways. It plays a role in both the cytokine-cytokine receptor interaction pathway and the NF-kB signaling pathway which are essential for immune response and cell signaling. Through these pathways it interacts with proteins like ICAM1 and various chemokines helping to coordinate the immune cell movement through vascular tissue barriers. |
| **Associated diseases and disorders** | Abnormal regulation of VCAM1 links to various cardiovascular diseases such as atherosclerosis and inflammatory diseases like rheumatoid arthritis. The protein levels may increase in these conditions promoting excessive immune cell recruitment and tissue damage. VCAM1 also interfaces with other proteins like ICAM1 in these pathological states which together contribute to the progression and severity of these diseases. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

We do not recommend this combination. It is not covered by our product promise.

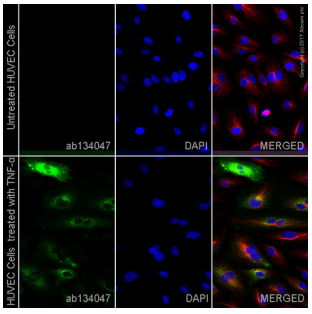
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We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

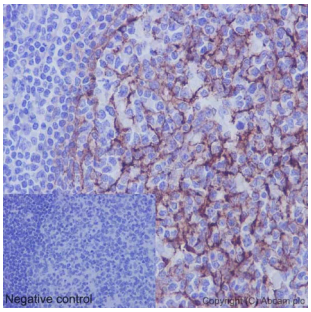
## 16 product images




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

VCAM1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-VCAM1 antibody

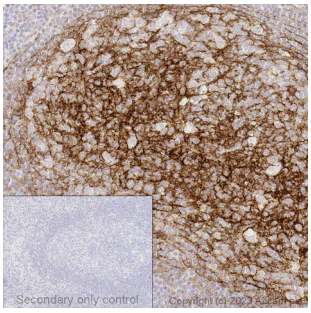
ab134047 staining VCAM1 in HUVEC cells treated with TNF-α (ab55237) at 10 ng/ml for 16 hours. The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells 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).




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

VCAM1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-VCAM1 antibody

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.

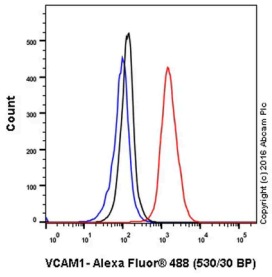



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

VCAM1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human tonsil using rabbit Anti-VCAM1 antibody

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.

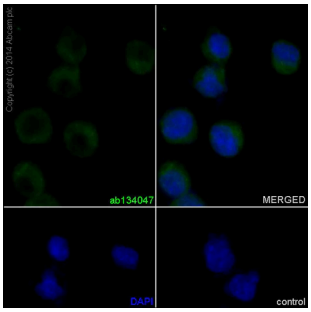
This data was developed using the same antibody clone in a different buffer formulation (ab271899).




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

VCAM1 Flow Cytometry (Intracellular) staining using rabbit Anti-VCAM1 antibody

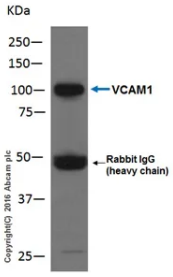
Intracellular Flow Cytometry analysis of K562 (human chronic myelogenous leukemia) cells labeling VCAM1 with purified ab134047 at 1/40 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor®488) at 1/2000 dilution was 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)

VCAM1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-VCAM1 antibody

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).




## 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.

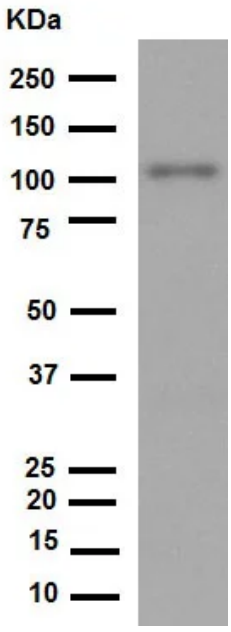
**Exposure time:** 1 second.

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

All lanes:
Immunoprecipitation - Anti-VCAM1 antibody [EPR5047] (ab134047)

Predicted band size: 81 kDa

Exposure time: 1s




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

VCAM1 Western blot staining of Human fetal liver tissue lysate using rabbit Anti-VCAM1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134047).
**Blocking buffer:** 5% NFDM/TBST.

**Dilution buffer:** 5% NFDM/TBST.

Lane 1:
Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/10000 dilution

Lane 1:
Western blot - Anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free (ab215380)

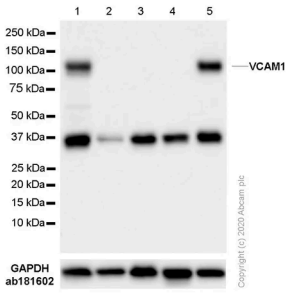
All lanes:
Human fetal liver tissue lysate at 20 µg

Secondary

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

Predicted band size: 81 kDa

Observed band size: 100 kDa




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

VCAM1 Western blot staining using rabbit Anti-VCAM1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134047).
Rabbit monoclonal [EPR16891] to GAPDH (ab181602) used as loading control.

Blocking/Diluting buffer: 5% NFDM/TBST.

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

Lane 1:
Hut-78 (Human Sezary syndrome cutaneous T lymphocyte) whole cell lysate at 20 µg

Lane 2:
SK-OV-3 (Human ovarian cancer epithelial cell) whole cell lysate at 20 µg

Lane 3:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 4:
J774A.1 (Mouse reticulum cell sarcoma monocyte macrophage) whole cell lysate at 20 µg

Lane 5:
LADMAC (Mouse bone marrow monocyte macrophage) whole cell lysate at 20 µg

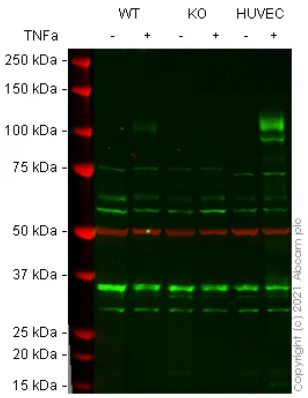
Secondary

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

Predicted band size: 81 kDa

Observed band size: 100 kDa, 36 kDa

Exposure time: 7s




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

VCAM1 Western blot staining using rabbit Anti-VCAM1 antibody

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.

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

Lane 1:
Wild-type A549 cell lysate at 30 µg

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

Lane 3:
VCAM1 knockout A549 cell lysate at 30 µg

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

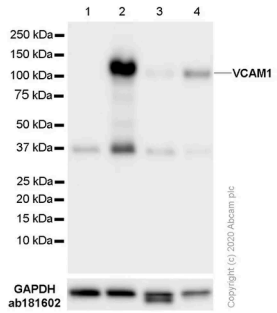
Lane 5:
HUVEC cell lysate at 30 µg

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

Performed under reducing conditions.

Predicted band size: 81 kDa

Observed band size: 105 kDa




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

VCAM1 Western blot staining using rabbit Anti-VCAM1 antibody

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

All lanes:

Western blot - Anti-VCAM1 antibody [EPR5047] (ab134047) at 1/1000 dilution

Lane 1:
HUVEC (Human umbilical vein endothelial cell) whole cell lysate at 20 µg

Lane 2:
HUVEC (Human umbilical vein endothelial cell) treated with 10 ng/ml TNF-α for 16 h, whole cell lysate at 20 µg

Lane 3:
bEnd.3 (Mouse brain endothelioma) whole cell lysate at 20 µg

Lane 4:
bEnd.3 (Mouse brain endothelioma) treated with 10 µg/ml LPS for 24 h, whole cell lysate at 20 µg

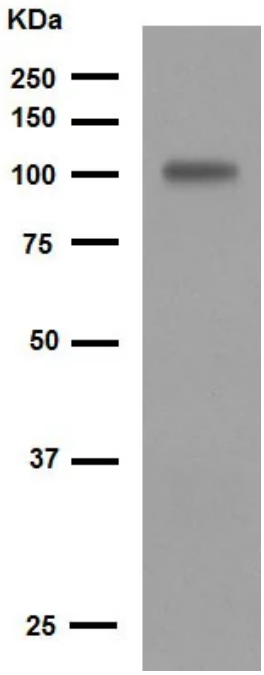
Secondary

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

Predicted band size: 81 kDa

Observed band size: 100 kDa, 36 kDa

Exposure time: 2s




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

VCAM1 Western blot staining of HuT-78 cell lysate using rabbit Anti-VCAM1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134047).
**Blocking buffer:** 5% NFDM/TBST.

**Dilution buffer:** 5% NFDM/TBST.

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

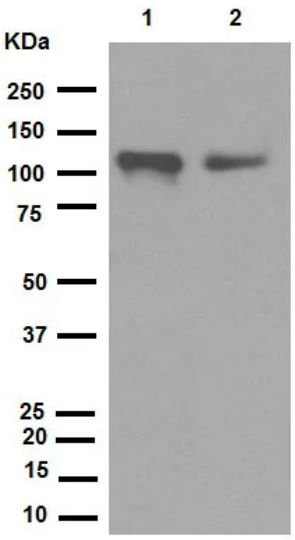
All lanes:
HuT-78 cell lysate at 10 µg

Secondary

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

Predicted band size: 81 kDa

Observed band size: 100 kDa




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

VCAM1 Western blot staining using rabbit Anti-VCAM1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134047).
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

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

Lane 1:
Mouse kidney at 20 µg

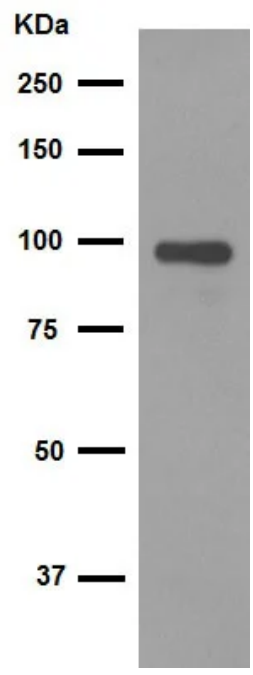
Lane 2:
Rat spleen at 20 µg

Secondary

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

Predicted band size: 81 kDa

Observed band size: 100 kDa




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

VCAM1 Western blot staining of NIH/3T3 cell lysate using rabbit Anti-VCAM1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134047).
**Blocking buffer:** 5% NFDM/TBST.

**Dilution buffer:** 5% NFDM/TBST.

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

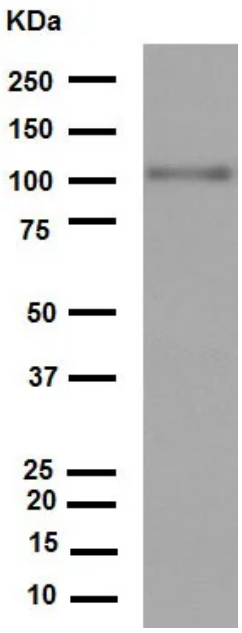
All lanes:
NIH/3T3 cell lysate at 10 µg

Secondary

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

Predicted band size: 81 kDa

Observed band size: 100 kDa




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

VCAM1 Western blot staining of Rat kidney tissue lysate using rabbit Anti-VCAM1 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab134047).
**Blocking buffer:** 5% NFDM/TBST.

**Dilution buffer:** 5% NFDM/TBST.

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

All lanes:
Rat kidney tissue lysate at 20 µg

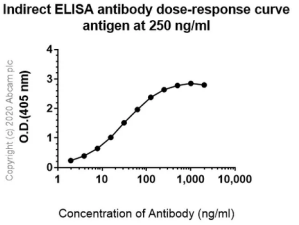
Secondary

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

Predicted band size: 81 kDa

Observed band size: 100 kDa

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

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

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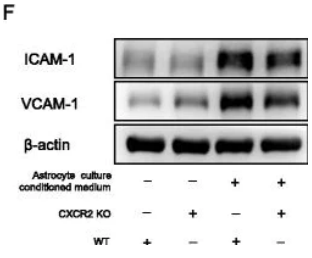



Image collected and cropped by CiteAb under a CC-BY license from the publication

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

VCAM1 Western blot staining using rabbit Anti-VCAM1 antibody

VCAM1 western blot using anti-VCAM1 antibody [EPR5047] - Low endotoxin, Azide free ab215380. Publication image and figure legend from Wu, F., Zhao, Y., et al., 2015, J Neuroinflammation, PubMed 25990934.

ab215380 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab215380 please see the product overview.

Astrocyte-derived CXCL1 and endothelial CXCR2 are essential for cerebral endothelial activation. A i.c.v. LPS injection (4 h) induced significant CXCR2 mRNA expression in WT mice. B Levels of CXCR2 protein after i.c.v. LPS injection from 4 to 24 h gradually increased compared with the control group (4 h after i.c.v. saline injection). C The expression of CXCR2 mRNA in primary brain microvascular endothelial cells, microglia, and astrocytes stimulated with either vehicle or TNF-α (100 ng/ml) was measured via real-time PCR. The results are represented as the means ± SEM of three independent experiments; *p < 0.05. D Primary endothelial cells, astrocytes, and microglia

were seeded at 2 × 106 cells/well in six-well plates and were incubated overnight. The following day, the cells were stimulated with 100 ng/ml LPS or 100 ng/ml TNF-α for 12 h. Cell lysates were collected and analyzed for CXCR2 expression via Western blot. E Primary astrocytes and microglia from wild-type mice were seeded at 2 × 106 cells/well in six-well plates and were incubated overnight. The following day, the cells were stimulated with 100 ng/ml LPS or 100 ng/ml TNF-α for 12 h. Then, the conditioned supernatants and cell lysates were collected and analyzed for CXCL1 expression via ELISA. The results are represented as the means ± SEM of three independent experiments; **p < 0.01. F Astrocyte culture conditioned medium was added into primary cerebral endothelial cells from wild-type or CXCR2-/- mice, and the levels of VCAM-1 and ICAM-1 were measured via Western blot

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