Anti-VCAM1 Antibody [SA05-04]

ET1601-18

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 81 kDa
Clone number:	SA05-04

Description: Vascular cell adhesion protein 1 also known as vascular cell adhesion molecule 1 (VCAM-1) or cluster of differentiation 106 (CD106) is a protein that in humans is encoded by the VCAM1 gene. VCAM-1 functions as a cell adhesion molecule. The VCAM-1 protein mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium. It also functions in leukocyte-endothelial cell signal transduction, and it may play a role in the development of atherosclerosis and rheumatoid arthritis.

Immunogen: Synthetic peptide within Human VCAM1 aa 690-739 / 739.

Positive control: C2C12 cell lysate, 3T3-L1 cell lysate, mouse spleen tissue lysate, rat spleen tissue lysate, NIH/3T3 cell lysate, HUVEC, human tonsil tissue, human spleen tissue, mouse spleen tissue.

Subcellular location: Membrane

Database links: SwissProt: P19320 Human | P29533 Mouse | P29534 Rat

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:1,000
IF-Tissue	1:100
IHC-P	1:200
IP	1-2μg/sample
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

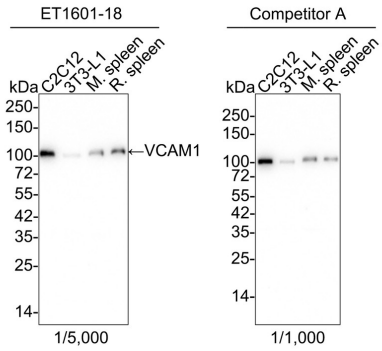

Fig1: Western blot analysis of VCAM1 on different lysates with Rabbit anti-VCAM1 antibody (ET1601-18) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: C2C12 cell lysate (20 µg/Lane)
Lane 2: 3T3-L1 cell lysate (20 µg/Lane)
Lane 3: Mouse spleen tissue lysate (20 µg/Lane)
Lane 4: Rat spleen tissue lysate (20 µg/Lane)

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

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1601-18) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

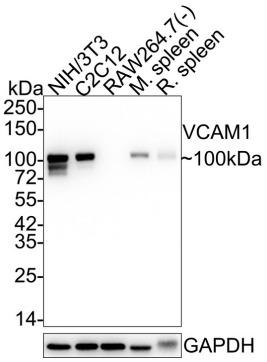

Fig2: Western blot analysis of VCAM1 on different lysates with Rabbit anti-VCAM1 antibody (ET1601-18) at 1/5,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: C2C12 cell lysate (20 µg/Lane)
Lane 3: RAW264.7 cell lysate (negative) (20 µg/Lane)
Lane 4: Mouse spleen tissue lysate (20 µg/Lane)
Lane 5: Rat spleen tissue lysate (20 µg/Lane)

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

Exposure time: 24 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1601-18) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

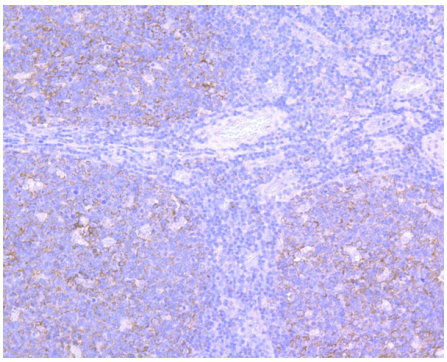

Fig3: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-VCAM1 antibody.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-18, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

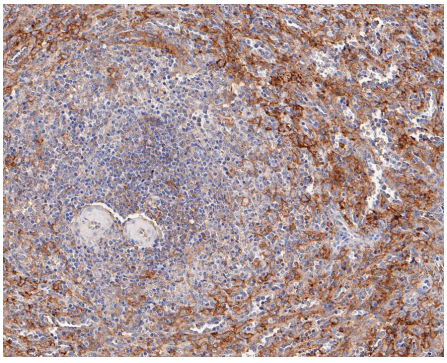

Fig4: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-VCAM1 antibody (ET1601-18) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-18) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

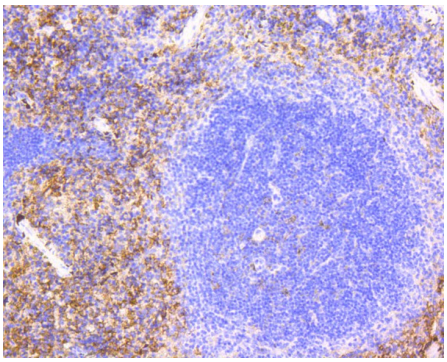

Fig5: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue using anti-VCAM1 antibody.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-18, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

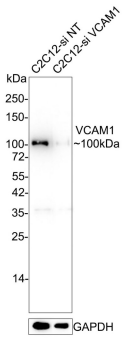

Fig6: Western blot analysis of VCAM1 on different lysates with Rabbit anti-VCAM1 antibody (ET1601-18) at 1/5,000 dilution.

Lane 1: C2C12-si NT cell lysate
Lane 2: C2C12-si VCAM1 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 1 minute; ECL: K1801;

4-20% SDS-PAGE gel.

ET1601-18 was shown to specifically react with VCAM1 in C2C12-si NT cells. Weakened band was observed when C2C12-si VCAM1 sample was tested. C2C12-si NT and C2C12-si VCAM1 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1601-18, 1/5,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

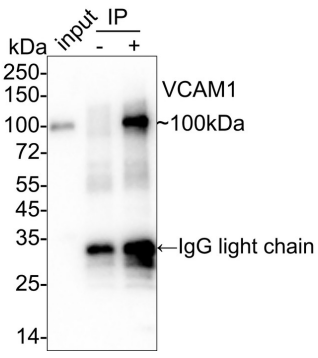

Fig7: VCAM1 was immunoprecipitated in 0.2mg C2C12 cell lysate with ET1601-18 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1601-18 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: C2C12 cell lysate (input)
Lane 2: Rabbit IgG instead of ET1601-18 in C2C12 cell lysate
Lane 3: ET1601-18 IP in C2C12 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 5 seconds; ECL: K1802

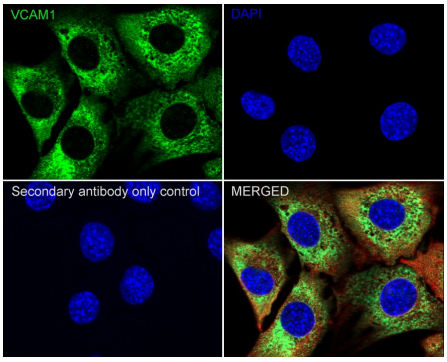

Fig8: Immunocytochemistry analysis of C2C12 cells labeling VCAM1 with Rabbit anti-VCAM1 antibody (ET1601-18) at 1/1,000 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-VCAM1 antibody (ET1601-18) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

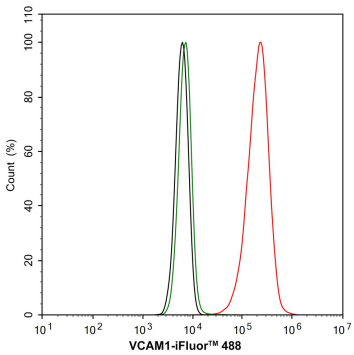

Fig9: Flow cytometric analysis of C2C12 cells labeling VCAM1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-18, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Gregory EK et al. Periadventitial atRA citrate-based polyester membranes reduce neointimal hyperplasia and restenosis after carotid injury in rats. Am J Physiol Heart Circ Physiol 307:H1419-29 (2014).
2. Florea V et al. c-Myc Is Essential to Prevent Endothelial Pro-Inflammatory Senescent Phenotype. PLoS One 8:e73146 (2013).