

APA547Hu61 100µg
Active Vascular Cell Adhesion Molecule 1 (VCAM1)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Gln109~Ser318

Tags: N-terminal His-tag

Purity: >92%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 5% trehalose.

Original Concentration: 650µg/mL

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 5.5

Predicted Molecular Mass: 25.0kDa

Accurate Molecular Mass: 30kDa as determined by SDS-PAGE reducing conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

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QV EIYSFPKDPE IHLGPLEAG KPITVKCSVA DVYPFDRLEI
DLLKGDHLMK SQEFLADR KSLETKSLEV TFTPVIIDIG KVLVCRALH
IDEMDSVPTV RQAVKELQVY ISPKNTVISV NPSTKLQEGG SVTMTCSSEG
LPAPEIFWSK KLDNGNLQHL SGNATLTLIA MRMEDSGIYV CEGVNLIGKN
RKEVELIVQE KPFTVEIS
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[ACTIVITY]

Vascular cell adhesion protein 1 (VCAM-1) is a cell adhesion molecule and a member of the immunoglobulin superfamily. Alternatively spliced forms are known to occur, but the most common form is a type I transmembrane protein with a 674 amino acid extracellular segment that includes seven C2-type immunoglobulin domains. VCAM-1 is expressed by activated endothelial cells and certain other cell types including macrophages, dendritic cells, neurons, smooth muscle cells, fibroblasts, and oocytes. Besides, Integrin Alpha 4 (ITGa4) has been identified as an interactor of VCAM-1, thus a binding ELISA assay was conducted to detect the interaction of recombinant human VCAM-1 and recombinant human ITGa4. Briefly, biotin-linked VCAM-1 were diluted serially in PBS, with 0.01% BSA (pH 7.4).

Duplicate samples of 100 µl were then transferred to ITGa4-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and

washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50 µl stop solution to the wells and read at 450nm immediately. The binding activity of recombinant human VCAM-1 and recombinant human ITGa4 was shown in Figure 1, the EC50 for this effect is 0.261 µg/mL.

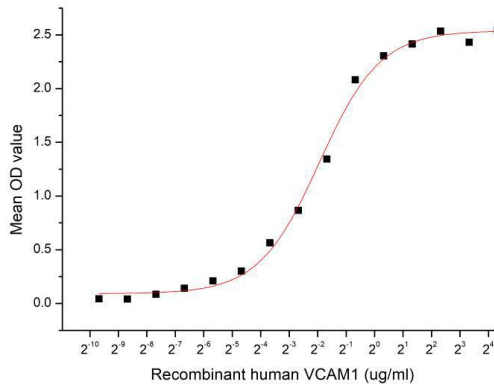


Figure 1. The binding activity of recombinant human VCAM-1 and recombinant human ITGa4

As VCAM1 has the function of cell adhesion, we measure the activity of VCAM1 by the ability of the immobilized protein to support the adhesion of U937 human histiocytic lymphoma cells. When 5 x 10⁴ cells/well are added to recombinant human VCAM1 coated plates (2.5 µg/mL with 100 µL/well), approximately 80-90% will adhere after 3 hour incubation at 37°C. The adhesion of U937 after 3 hour incubation at 37°C observed by inverted microscope was shown in Figure 2.

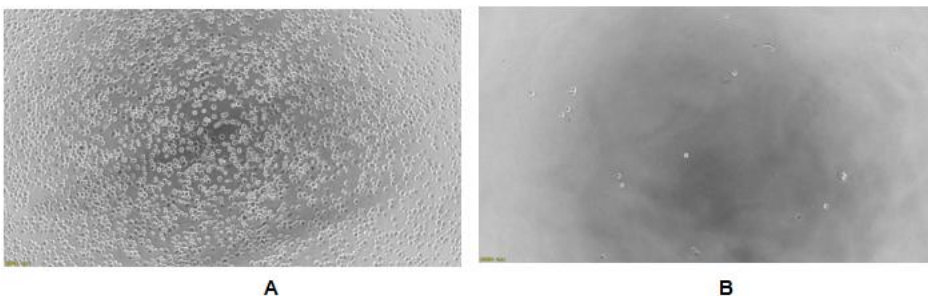


Figure 2. The adhesion of U937 supported by recombinant human VCAM1

- (A) U937 cultured in recombinant human VCAM1 coated plates (10 µg/mL with 100 µL/well);
- (B) U937 cultured in without-protein coated plates.

