Quantikine® ELISA

Human sVCAM-1/CD106 Immunoassay

Catalog Number DVC00
SVC00
PDVC00

For the quantitative determination of human soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Human Vascular Cell Adhesion Molecule-1 (VCAM-1) is a 100-110 kDa, 715 amino acid (aa) type I transmembrane glycoprotein typically characterized by the presence of seven C2-type immunoglobulin (Ig) domains (1-3). Its extracellular region is 674 aa in length, followed by a 22 aa transmembrane segment and a 19 aa cytoplasmic tail (1, 2). In the extracellular region, there are multiple N-linked glycosylation sites (the predicted molecular weight is 80 kDa), and each C2 domain is closed by a disulfide bridge. There is considerable interspecies VCAM-1 homology, with mouse and rat VCAM-1 showing approximately 75% aa identity to human VCAM-1 (2-4). Notably, the short 19 aa cytoplasmic tail is absolutely conserved, mouse to human to rat (4). Cells expressing mouse VCAM-1 bind both mouse and human leukocytes, and this reflects their high degree of aa identity (4). A number of variants of VCAM-1 are known to occur, all of which are likely the result of alternate gene splicing. In particular, a human six Ig domain molecule is known (1), and in rabbits, an eight Ig domain form has been identified (2). There is also a three-C2 domain, 43 kDa GPI-linked form of VCAM-1 (5, 6). Although it binds known VCAM-1 ligands (or co-receptors), its function is unclear. Cells known to express VCAM-1 include neurons (7), endothelial cells (8), smooth muscle cells (9), fibroblasts (10) and macrophages (11).

Soluble VCAM-1 has been identified in culture supernates (12), blood (13-15), and cerebrospinal fluid (15, 16). *In vitro*, basal levels of VCAM-1 shedding by unstimulated NIH-3T3 cells appear to partially require metalloproteinase activity, while PMA-induced shedding is dependent upon the proteolytic activity of TACE/ADAM17 (12).

Functionally, VCAM-1 binds to both α4β1 (VLA-4) and α4β7 (LPAM-1) integrins (17, 18). These integrins (or VCAM-1 ligands) are expressed on a variety of cells, with VLA-4 found on all leukocytes with the exception of neutrophils (17, 19, 20). Because of this, VCAM-1/VCAM-1 ligand interactions are undoubtedly key events in the rate and timing of leukocyte extravasation (3). Other roles proposed for VCAM-1 include the regulation of osteoclastogenesis via a cell-to-cell contact mechanism (22) and the induction of sickle cell adherence to vascular endothelial cells during hypoxemia (23).

The Quantikine Human sVCAM-1/CD106 Immunoassay is a 2 hour solid-phase ELISA designed to measure human sVCAM-1 in cell culture supernates, serum, and plasma. It contains CHO cell-expressed recombinant human sVCAM-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human sVCAM-1 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring sVCAM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sVCAM-1has been pre-coated onto a microplate. Standards, samples, controls, and conjugate are pipetted into the wells and any sVCAM-1 present is sandwiched by the immobilized antibody and the enzyme-linked monoclonal antibody specific for sVCAM-1. Following a wash to remove any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of sVCAM-1 bound. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DVC00	CATALOG # SVC00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
sVCAM-1 Microplate	892717	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human sVCAM-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
sVCAM-1 Conjugate	892718	1 vial	6 vials	11 mL/vial of a monoclonal antibody against sVCAM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
sVCAM-1 Standard	892719	1 vial	6 vials	400 ng/vial of recombinant human sVCAM-1 in a buffer with preservatives; lyophilized.	
Calibrator Diluent RD5P	895151	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DVC00 contains sufficient materials to run an ELISA on one 96 well plate.
SVC00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDVC00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human sVCAM-1 Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

All samples require a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calbrator Diluent RD5P (1X).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

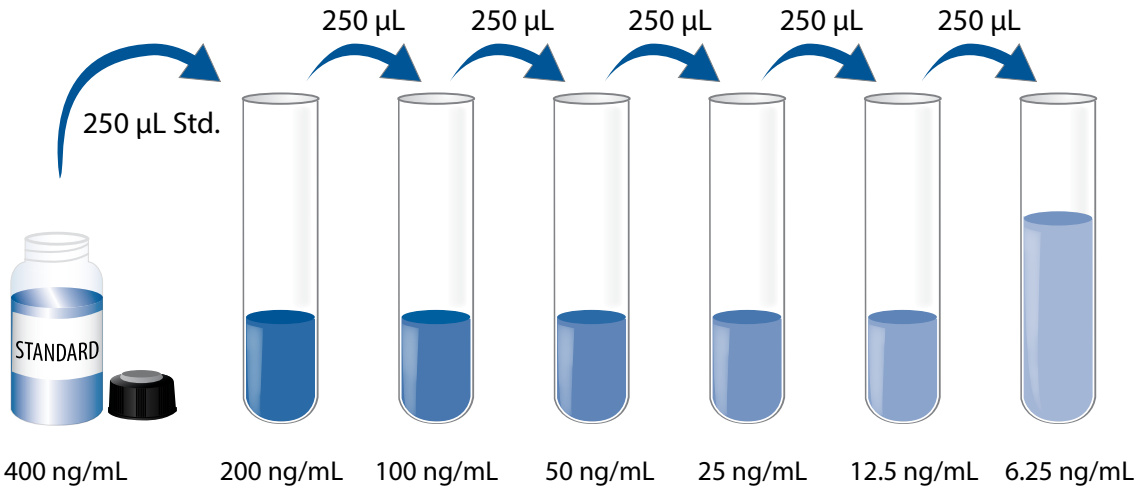
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (1X) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (1X).

sVCAM-1 Standard - Reconstitute the sVCAM-1 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 400 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 250 µL of Calibrator Diluent RD5P (1X) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (1X) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of sVCAM-1 Conjugate to each well.
4. Add 100 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Immediately add 100 µL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 20 minutes at room temperature. **Protect from light.**
7. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

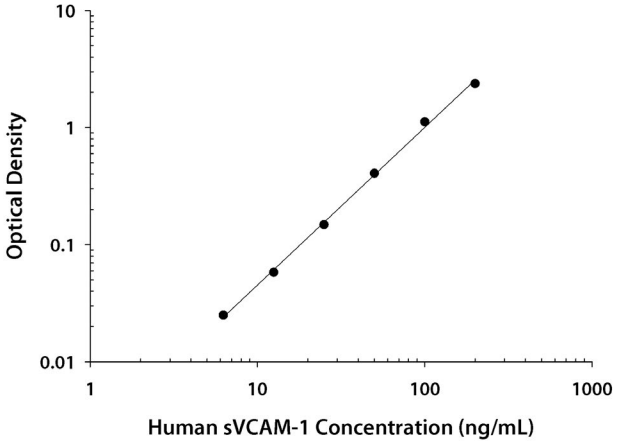
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the sVCAM-1 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.037 0.039	0.038	—
6.25	0.062 0.064	0.063	0.025
12.5	0.096 0.096	0.096	0.058
25	0.185 0.187	0.186	0.148
50	0.443 0.446	0.445	0.407
100	1.141 1.172	1.157	1.119
200	2.361 2.434	2.413	2.375

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	583	1423	2421	670	1571	2726
Standard deviation	13.6	49.5	86.4	52.1	120.9	151.2
CV (%)	2.3	3.5	3.6	7.8	7.7	5.5

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of sVCAM-1 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media* (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	106	106	105	105
	Range (%)	104-109	105-108	103-107	103-108
1:4	Average % of Expected	96	97	95	96
	Range (%)	87-101	90-102	87-99	89-101
1:8	Average % of Expected	109	111	106	109
	Range (%)	98-114	102-115	99-110	101-113
1:16	Average % of Expected	88	96	89	91
	Range (%)	86-90	93-99	86-93	88-93

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of sVCAM-1 ranged from 0.17-1.26 ng/mL. The mean MDD was 0.6 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human VCAM-1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of sVCAM-1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	557	349-991	139.6
EDTA plasma (n=36)	531	341-897	132.5
Heparin plasma (n=36)	491	301-875	149.3

Cell Culture Supernates - Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural sVCAM-1. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human sVCAM-1.

The factors listed below were prepared at 617 ng/mL in Calibrator Diluent RD5P (1X) and assayed for cross-reactivity. Preparations of the following factors at 617 ng/mL in a mid-range recombinant human sVCAM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ALCAM/CD166
BCAM
CAD-8
Contactin-1
DNAM-1
Desmoglein-1
Desmoglein-2
ECAD
Ecalectin
E-Selectin
ICAM-1
ICAM-2
ICAM-3
ICAM-5
JAM-1
JAM-2
JAM-3
LAMP
L-Selectin
MCAM
NCAD
NCAM-L1
PCAD
PECAM-1/CD31
P-Selectin
TROP-2
VE-CAD

Recombinant mouse:

ALCAM/CD166
ECAD
E-Selectin
ICAM-1
ICAM-2
ICAM-5
JAM-1
JAM-2
JAM-3
L-Selectin
PCAD
P-Selectin
VCAM-1/CD106

REFERENCES

1. Osborn, L. *et al.* (1989) Cell **59**:1203.
2. Cybulsky, M.I. *et al.* (1991) Proc. Natl. Acad. Sci. USA **88**:7859.
3. Hession, C. *et al.* (1991) J. Biol. Chem. **266**:6682.
4. Hession, C. *et al.* (1992) Biochem. Biophys. Res. Commun. **183**:163.
5. Moy, P. *et al.* (1993) J. Biol. Chem. **268**:8835.
6. Terry, R.W. *et al.* (1993) Proc. Natl. Acad. Sci. USA **90**:5919.
7. Birdsall, H.H. *et al.* (1992) J. Immunol. **148**:2717.
8. Sano, H. *et al.* (1995) Int. Arch. Allergy Immunol. **107**:533.
9. Ardehali, A. *et al.* (1995) Circulation **92**:450.
10. Meng, H. *et al.* (1995) J. Invest. Dermatol. **105**:789.
11. van Oosten, M. *et al.* (1995) Hepatology **22**:1538.
12. Garton, K.J. *et al.* (2003) J. Biol. Chem. **278**:37459.
13. Duits, A.J. *et al.* (1996) Clin. Immunol. Immunopathol. **81**:96.
14. Sudhoff, T. *et al.* (1996) Leukemia **10**:682.
15. Matsuda, M. *et al.* (1995) J. Neuroimmunol. **59**:35.
16. Droogan, A.G. *et al.* (1996) J. Neuroimmunol. **64**:185.
17. Rott, L.S. *et al.* (1996) J. Immunol. **156**:3727.
18. Chan, B.M.C. *et al.* (1992) J. Biol. Chem. **267**:8366.
19. Hemler, M.E. (1990) Annu. Rev. Immunol. **8**:365.
20. Bochner, B.S. *et al.* (1991) J. Exp. Med. **173**:1553.
21. Imhof, B.A. and D. Dunon (1995) Adv. Immunol. **58**:345.
22. Feuerbach, D. and J.H.M. Feyen (1997) FEBS Lett. **402**:21.
23. Setty, B.N.Y. and M.J. Stuart (1996) Blood **88**:2311.