Quantikine®

Human sVCAM-1 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 NIH3T3 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 $\alpha_4\beta_1$ (VLA-4) and $\alpha_4\beta_7$ (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 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 the Quantikine Human sVCAM-1 immunoassay 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 the 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.
- 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.

MATERIALS PROVIDED

Description	Part #	Cat. # DVC00	Cat. # SVC00
sVCAM-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human sVCAM-1.	892717	1 plate	6 plates
sVCAM-1 Conjugate - 11 mL/vial of monoclonal antibody against sVCAM-1 conjugated to horseradish peroxidase with preservatives.	892718	1 vial	6 vials
sVCAM-1 Standard - 400 ng/vial of recombinant human sVCAM-1 in a buffer with preservatives; lyophilized.	892719	1 vial	6 vials
Calibrator Diluent RD5P Concentrate - 21 mL/vial of a concentrated buffered protein base with preservatives.	895151	1 vial	6 vials
Wash Buffer Concentrate - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
Stop Solution - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
Plate Covers - Adhesive strips.	—	4 strips	24 strips

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.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Calibrator Diluent RD5P	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

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

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.
- Human sVCAM-1 Controls (optional; available from R&D Systems).

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

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 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 heparin or EDTA 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 sample + 380 μL Calbrator Diluent RD5P (1X).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

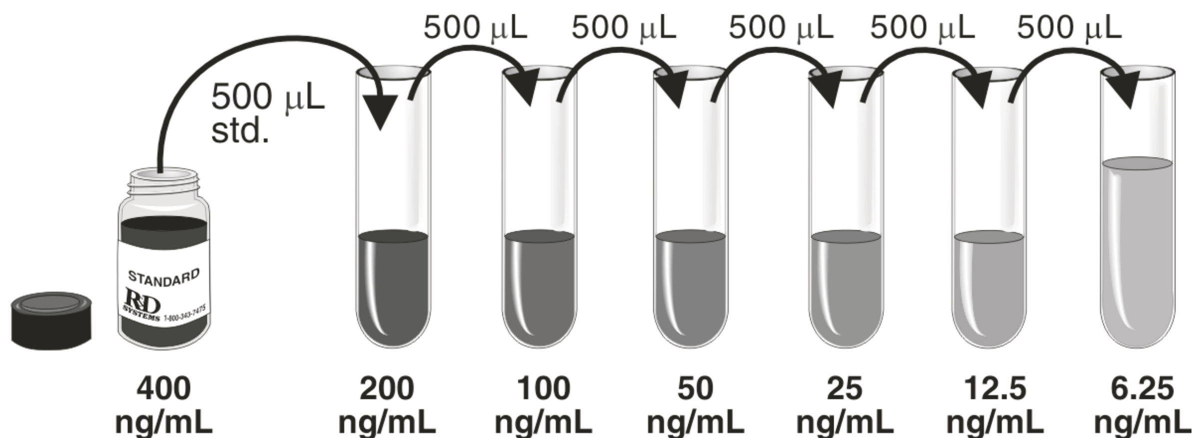
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

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) - Dilute 20 mL of Calibrator Diluent RD5P Concentrate into 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 500 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

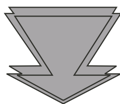
Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

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. A plate layout is provided to record standards and samples assayed.
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.

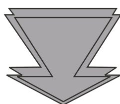
*Samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

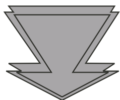
1. Prepare reagents, Standards, and samples as instructed.



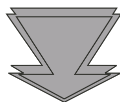
2. Add 100 µL of sVCAM-1 Conjugate to each well.



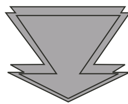
3. Add 100 µL Standard, control, or sample* to each well. Incubate 1.5 hours at RT.



4. Aspirate and wash 4 times.



5. Add 100 µL Substrate to each well. Incubate for 20 minutes at RT.
Protect from light.



6. Add 50 µL Stop Solution to each well. Read at 450 nm within 30 minutes.
λ correction 540 nm

*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.

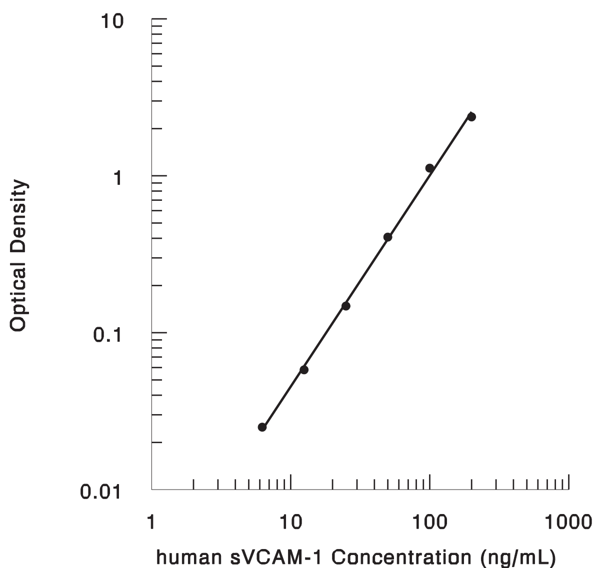
Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

To determine the sVCAM-1 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding sVCAM-1 concentration.

Since the 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

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.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.

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 the Calibrator Diluent RD5P (1X) to produce samples with values within the dynamic range of the assay.

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

*Samples were first diluted 20-fold 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 drawn 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 (1×10^6 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 culture supernate were removed and assayed for levels of natural sVCAM-1. No detectable levels were observed.

SPECIFICITY

This assay recognizes recombinant and natural 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

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PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

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