Instructions for Use

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sVCAM-1 ELISA

Enzyme-linked immunosorbent assay
for the quantitative detection of human VCAM-1

REF **30150479**

Σ **96**

2°C 8°C

For research use only.
Not for use in diagnostic procedures.

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Always there for you

1 INTENDED USE

The sVCAM-1 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human VCAM-1. For research use only. Not for diagnostic or therapeutic procedures.

2 SUMMARY

The vascular cell adhesion molecule-1 (VCAM-1) or CD106 is a member of the immunoglobulin gene superfamily. The initial molecular cloning of VCAM-1 reported six extracellular Ig-like domains (6D VCAM-1).

This 6D VCAM-1 arises due to alternative splicing from a seven-domain VCAM-1 (7D VCAM-1). 7D VCAM-1 is the dominant form expressed by cultured human endothelial cells. Domains 1 through 3 are highly homologous to domains 4 through 6, suggesting that they arose by gene duplication. The cDNA of 7D VCAM-1 predicts a core protein of approximately 81 kDa with seven potential N-linked glycosylation sites. Upon complete glycosylation the mature protein has a molecular weight of approximately 102 kDa. This observation is in general agreement with immunoprecipitation studies that show a protein of approximately 110 kDa on cytokine-activated endothelium.

Murine and rat VCAM-1 have been cloned. In contrast to ICAM-1, VCAM-1 appears to have been highly conserved through evolution. Both rat and mouse VCAM-1 are highly homologous at the protein level to the human VCAM-1 (77% and 76%, respectively). VCAM-1 supports the adhesion of lymphocytes, monocytes, natural killer cells, eosinophils, and basophils through its interaction with leukocyte very late antigen-4 (VLA-4). VCAM-1/VLA-4 interaction mediates firm adherence of circulating non-neutrophilic leukocytes to endothelium. VCAM-1 also participates in leukocyte adhesion outside of the vasculature, mediating precursor lymphocyte adhesion to bone marrow stromal cells and B cell binding to lymph node follicular dendritic cells.

VCAM-1 is not constitutively expressed on endothelium, but can be up-regulated in vitro in response to LPS, TNF alpha, and IL-1, as well as to interferon gamma and IL-4.

VCAM-1 is also present on tissue macrophages, dendritic cells, bone marrow fibroblasts, myoblasts and myotubes.

3 PRINCIPLES OF THE TEST

An anti-human VCAM-1 coating antibody is adsorbed onto microwells.

Figure 1

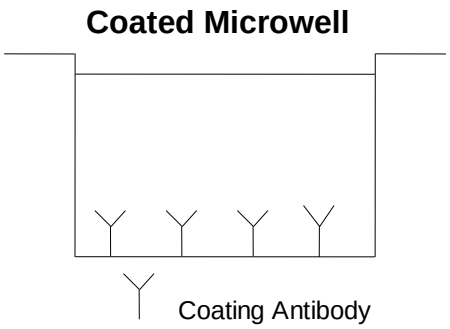


Figure 2

Human VCAM-1 present in the sample or standard binds to antibodies adsorbed to the microwells. A conjugate mixture (biotin-conjugated anti-human VCAM-1 antibody and Streptavidin-HRP) is added.

Biotin-conjugated anti-human VCAM-1 antibody binds to human VCAM-1 captured by the first antibody.

Streptavidin-HRP binds to the biotin-conjugated anti-human VCAM-1 antibody.

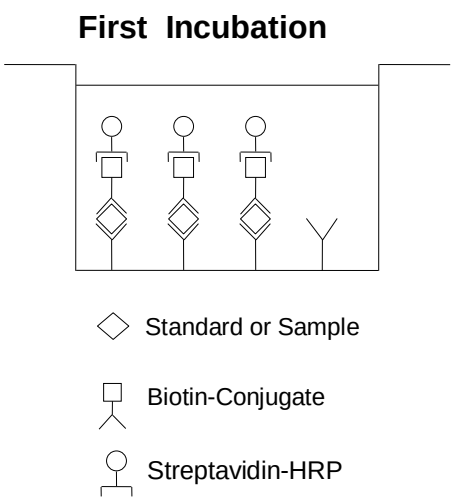


Figure 3

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

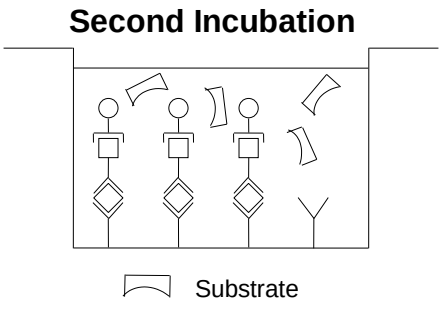
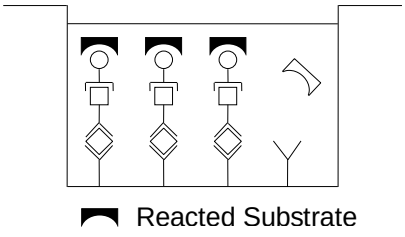


Figure 4

A colored product is formed in proportion to the amount of human VCAM-1 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 6 human VCAM-1 standard dilutions and human VCAM-1 sample concentration determined.



4 REAGENTS PROVIDED

MTP	1 aluminium pouch with a **Microtiter Plate** (12 strips with 8 wells each) coated with monoclonal antibody to human VCAM-1
CONJMIX **CONC**	1 vial (100 µL) **Conjugate Mixture** containing **Biotin-Conjugate** anti-human VCAM-1 monoclonal antibody mixed with **Streptavidin-HRP**
CAL **LYO**	2 vials human VCAM-1 **Standard** lyophilized, 200 ng/mL upon reconstitution
CONTROL H **LYO**	1 vial **Control high,** lyophilized
CONTROL L **LYO**	1 vial **Control low,** lyophilized
ASSAYBUF **CONC**	1 vial (5 mL) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20, 10% BSA)
WASHBUF **CONC**	1 bottle (50 mL) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)
SUBS	1 vial (15 mL) **Substrate Solution** (tetramethyl-benzidine)
STOP	1 vial (15 mL) **Stop Solution** (1M Phosphoric acid)
	2 **Adhesive Films**

5 STORAGE INSTRUCTIONS – ELISA KIT

Store kit reagents between 2°C and 8°C except controls. Store lyophilized controls at -20°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C), controls to -20°C, respectively. Expiry of the kit and reagents is stated on labels.
Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6 SAMPLES COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum, plasma (EDTA, citrate, heparin) and amniotic fluid were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human VCAM-1. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to “Sample stability“).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

8 PRECAUTIONS FOR USE

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 PREPARATION OF REAGENTS

1. Buffer concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

9.1 Wash Buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2°C to 25°C. Note that Wash Buffer (1x) is stable for 30 days.
4. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

9.2 Assay Buffer (1x)

1. Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
2. Store at 2°C to 8°C. Note that the Assay Buffer (1x) is stable for 30 days.
3. Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

9.3 Conjugate Mixture

Note: The Conjugate Mixture should be used within 30 minutes after dilution.

1. The Conjugate Mixture (Biotin-Conjugate mixed with Streptavidin-HRP) must be diluted 1:100 with Assay Buffer (1x) just prior to use in a clean plastic test tube.
2. Conjugate Mixture may be prepared as needed according to the following table:

Number of Strips	Conjugate Mixture (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

9.4 Human VCAM-1 Standard

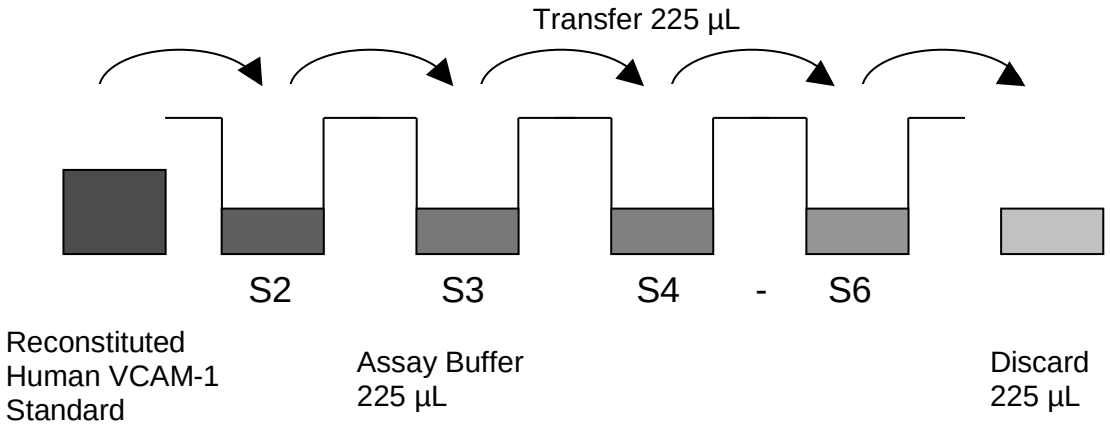
1. Reconstitute human VCAM-1 standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 200 ng/mL).
2. Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
3. After usage remaining standard cannot be stored and has to be discarded.
4. Standard dilutions can be prepared directly on the microwell plate (see “Test protocol“) or alternatively in tubes (see “External standard dilution“).

9.4.1 External Standard Dilution

1. Label 6 tubes, one for each standard point. S1, S2, S3, S4, S5, S6
2. Prepare 1:2 serial dilutions for the standard curve as follows:
   Pipette 225 µl of Assay Buffer (1x) into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 200 ng/mL) into the first tube, labelled S1, and mix (concentration of standard 1 = 100 ng/mL).
4. Pipette 225 µL of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 4 more times thus creating the points of the standard curve (see Figure 5).

   Assay Buffer (1x) serves as blank.

Figure 5



9.5 Controls

Reconstitute lyophilized controls by addition of distilled water (10-30 minutes). Reconstitution volume is stated on the label of the control vial. Swirl or mix gently to ensure complete and homogeneous solubilization. Further treat the controls like your samples in the assay. For control range please refer to certificate of analysis. Store reconstituted controls aliquoted at -20°C. Avoid repeated freeze and thaw cycles.

10 TEST PROTOCOL

a. Predilute your samples before starting with the test procedure. Dilute serum and plasma samples 1:50 with Assay Buffer (1x) according to the following scheme: 10 µL sample + 490 µL Assay Buffer (1x)

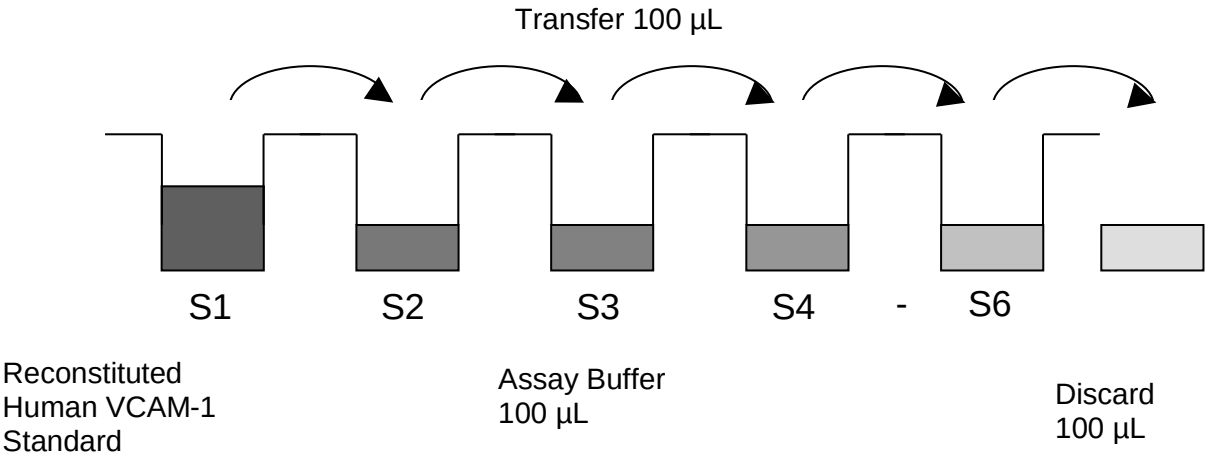
b. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control samples should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°C - 8°C sealed tightly.

c. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.
   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes - see “External standard dilution“):
   Add 100 µL of Assay Buffer (1x) in duplicate to all standard wells. Pipette 100 µL of prepared standard (see “Human VCAM-1 standard“, concentration = 200.0 ng/mL) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 100.0 ng/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 6). Take care not to scratch the inner surface of the microwells. Continue this procedure 4 times, creating

two rows of human VCAM-1 standard dilutions ranging from 100.0 to 3.1 ng/mL. Discard 100 µL of the contents from the last microwells (F1, F2) used.

Figure 6 Dilute standards - microwell plate.



In case of an external standard dilution (see "External standard dilution"), pipette 100 µL of these standard dilutions (S1 – S6) in the standard wells according to Table 1.

Table 1
Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (100.0 ng/mL)	Standard 1 (100.0 ng/mL)	Sample 2	Sample 2
B	Standard 2 (50.0 ng/mL)	Standard 2 (50.0 ng/mL)	Sample 3	Sample 3
C	Standard 3 (25.0 ng/mL)	Standard 3 (25.0 ng/mL)	Sample 4	Sample 4
D	Standard 4 (12.5 ng/mL)	Standard 4 (12.5 ng/mL)	Sample 5	Sample 5
E	Standard 5 (6.3 ng/mL)	Standard 5 (6.3 ng/mL)	Sample 6	Sample 6
F	Standard 6 (3.1 ng/mL)	Standard 6 (3.1 ng/mL)	Sample 7	Sample 7
G	Blank	Blank	Sample 8	Sample 8
H	Sample 1	Sample 1	Sample 9	Sample 9

e. Add 100 µL of Assay Buffer (1x) in duplicate to the blank wells.

f. Add 100 µL of each prediluted sample in duplicate to the sample wells.

g. Prepare Conjugate Mixture (refer to "Conjugate Mixture".)

h. Add 50 µL of diluted Conjugate Mixture to all wells, including the blank wells.

i. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker.

j. Remove adhesive film and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

k. Pipette 100 µL of TMB Substrate Solution to all wells.

l. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

m. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

n. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human VCAM-1 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human VCAM-1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human VCAM-1 concentration.
- If instructions in this protocol have been followed, serum and plasma samples have been diluted 1:50 (10 µL sample + 490 µL Assay Buffer (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 50).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human VCAM-1 levels. Such samples require further external predilution according to expected human VCAM-1 values with Assay Buffer (1x) in order to precisely quantitate the actual human VCAM-1 level.
- It is suggested that each testing facility establishes a control sample of known human VCAM-1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 7.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 7 Representative standard curve for human VCAM-1 ELISA. Human VCAM-1 was diluted in serial 2-fold steps in Assay Buffer (1x). Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

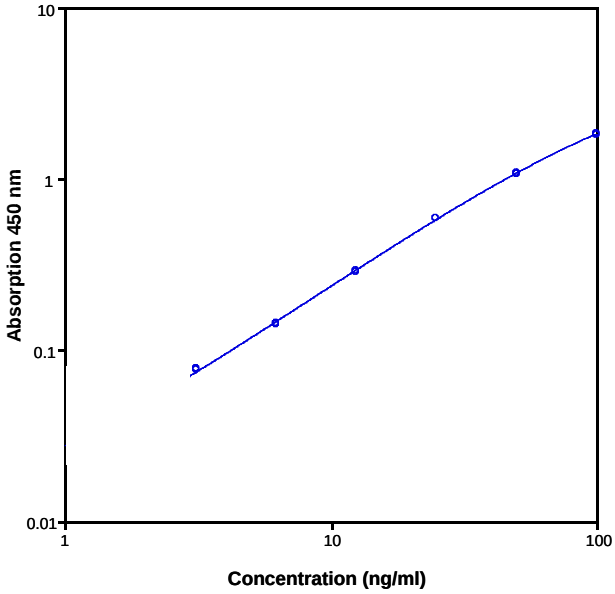


Table 2

Typical data using the sVCAM-1 ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human VCAM-1 Concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	100.0	1.808 1.837	1.823	0.8
2	50.0	1.080 1.058	1.069	1.0
3	25.0	0.582 0.583	0.583	0.1
4	12.5	0.284 0.288	0.286	0.7
5	6.3	0.144 0.141	0.142	1.1
6	3.1	0.077 0.078	0.078	0.6
Blank	0	0.032 0.028	0.030	6.7

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

12 LIMITATIONS

- Because exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of samples with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13 PERFORMANCE CHARACTERISTICS

13.1 Sensitivity

The limit of detection of human VCAM-1 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.6 ng/mL (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human VCAM-1. 2 standard curves were run on each plate. Data below show the mean human VCAM-1 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 3.1%.

Table 3 The mean human VCAM-1 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human VCAM-1 Concentration (ng/mL)	Coefficient of Variation (%)
1	1	1550.5	1.9
	2	1429.1	1.7
	3	1397.5	0.3
2	1	689.2	1.4
	2	698.3	2.6
	3	660.6	3.0
3	1	781.8	3.2
	2	808.7	1.5
	3	732.5	1.3
4	1	543.2	6.1
	2	575.5	2.4
	3	500.3	2.7
5	1	395.4	3.4
	2	461.1	7.4
	3	430.4	8.3
6	1	435.3	1.4
	2	458.4	3.3
	3	431.5	2.5
7	1	384.7	6.0
	2	412.1	2.0
	3	368.1	0.6
8	1	460.0	3.2
	2	498.6	2.8
	3	458.8	4.5

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human VCAM-1. 2 standard curves were run on each plate. Data below show the mean human VCAM-1 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 5.2%.

Table 4 The mean human VCAM-1 concentration and the coefficient of variation of each sample

Sample	Mean Human VCAM-1 Concentration (ng/mL)	Coefficient of Variation (%)
1	1459.0	5.5
2	682.7	2.9
3	774.3	5.0
4	539.7	7.0
5	428.9	7.7
6	441.7	3.3
7	388.3	5.7
8	472.5	4.8

13.3 Spike Recovery

The spike recovery was evaluated by spiking 7 levels of human VCAM-1 into pooled normal serum samples. Recoveries were determined in 3 independent experiments with 6 replicates each.
The amount of endogenous human VCAM-1 in unspiked serum was subtracted from the spike values.
The recovery ranged from 85% to 104% with an overall mean recovery of 89%.

13.4 Dilution Parallelism

Four serum samples with different levels of human VCAM-1 were analysed at serial 2 fold dilutions with 4 replicates each.
The recovery ranged from 87% to 120% with an overall recovery of 100% (see Table 5).

Table 5

Sample	Dilution	Expected Human VCAM-1 Concentration (ng/mL)	Observed Human VCAM-1 Concentration (ng/mL)	Recovery of Expected Human VCAM-1 Concentration (%)
1	1:50	--	1409.3	--
	1:100	704.6	639.5	91
	1:200	352.3	306.0	87
	1:400	176.2	166.2	94
2	1:50	--	639.1	--
	1:100	319.5	308.3	97
	1:200	159.8	174.2	109
	1:400	79.9	86.9	109
3	1:50	--	795.3	--
	1:100	397.7	368.2	93
	1:200	198.8	189.5	95
	1:400	99.4	94.5	95
4	1:50	--	508.8	--
	1:100	254.4	244.8	96
	1:200	127.2	152.7	120
	1:400	63.6	70.1	110

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human VCAM-1 levels determined. There was no significant loss of human VCAM-1 immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature and at 37°C, and the human VCAM-1 level determined after 24 hours. There was no significant loss of human VCAM-1 immunoreactivity detected during storage at -20°C, 2-8°C and room temperature.
A significant loss of human VCAM-1 immunoreactivity (20%) was detected during storage at 37°C after 24 hours.

13.6 Comparison of Serum and Plasma

From 2 individuals each, serum as well as EDTA, citrate, and heparin plasma was obtained at the same time and tested for human VCAM-1. Concentrations were not significantly different and therefore all these blood preparations are suitable for use in the assay.
It is nevertheless highly recommended to assure the uniformity of blood preparations.

13.7 Specificity

The assay detects both natural and recombinant human VCAM-1.
The interference of circulating factors of the immune systeme was evaluated by spiking these proteins at physiologically relevant concentrations into a human VCAM-1 positive serum.
No cross-reactivity was detected, notably not with other members of the immunoglobulin gene superfamily.

14 REAGENT PREPARATION SUMMARY

14.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20x (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

14.2 Assay Buffer (1x)

Add **Assay Buffer Concentrate 20x** (5 mL) to 95 mL distilled water.

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

14.3 Conjugate Mixture

Make a 1:100 dilution of the **Conjugate Mixture** (Biotin-Conjugate mixed with Streptavidin-HRP) in Assay Buffer (1x) just prior to use:

Number of Strips	Conjugate Mixture (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

14.4 Controls

Reconstitute lyophilized controls by addition of distilled water (10-30 minutes). Reconstitution volume is stated on the label of the control vial.

15 TEST PROTOCOL SUMMARY

1. Predilute serum and plasma samples with Assay Buffer (1x) 1:50.
2. Determine the number of microwell strips required.
3. Wash microwell strips twice with Wash Buffer.
4. Standard dilution on the microwell plate: Add 100 µL Assay Buffer, in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.
   Alternatively external standard dilution in tubes (see “External standard dilution“): Pipette 100 µL of these standard dilutions in the microwell strips.
5. Add 100 µL Assay Buffer (1x), in duplicate, to the blank wells.
6. Add 100 µL prediluted samples, in duplicate, to designated sample wells.
7. Prepare Conjugate Mixture.
8. Add 50 µL Conjugate Mixture to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
10. Empty and wash microwell strips 3 times with Wash Buffer.
11. Add 100 µL of TMB Substrate Solution to all wells.
12. Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
13. Add 100 µL Stop Solution to all wells.
14. Blank microwell reader and measure color intensity at 450 nm.

Note: If instructions in this protocol have been followed, serum and plasma samples have been diluted 1:50 (10 µL sample + 490 µL Assay Buffer (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 50).

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.	

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

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