KAMIYA BIOMEDICAL COMPANY

Human sVE-cadherin ELISA

For the quantitative determination of sVE-cadherin in cell culture supernatants, human serum, plasma or other body fluids

Cat. No. KT-031

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Human sVE-cadherin ELISA is an enzyme-linked immunosorbent assay for quantitative determination of soluble human vascular endothelial cadherin (VE-cadherin) in cell culture supernatants, human serum, plasma or other body fluids. For research use only. Not for use in diagnostic or therapeutic procedures.

DESCRIPTION
Cadherin-5, though a member of the family of cadherins, has been shown to be functionally and structurally distinct from classical cadherins (e.g. E-, N-, P-cadherins). Through its function and location cadherin-5 has been named VE-cadherin. It is a protein of a relative molecular mass of about 130 kDa.

VE-cadherin belongs to the group of adhesion molecules responsible for cellular interactions. The VE-cadherin gene encodes a Ca^{2+}-dependent cell adhesion molecule required for the organization of interendothelial junctions. This gene is exclusively and constitutively expressed in endothelial cells and the corresponding protein, an endothelial-specific cadherin, is localized at the intercellular junctions. VE-cadherin mediates homophilic, calcium-dependent aggregation and cell-to-cell adhesion. In addition, it decreases intercellular permeability to high-molecular weight molecules and reduces cell migration rate across a wounded area. Thus, VE-cadherin may exert a relevant role in endothelial cell biology through control of the cohesion and organization of the intercellular junctions.

The opening of the VE-cadherin mediated endothelial barrier may be a relevant step during neutrophil extravasation. This means that despite the fact that VE-cadherin is a “nonclassical” cadherin by structure, it functions as a classic cadherin.

Vascular endothelial growth factor (VEGF) stimulation results in a maximal tyrosine phosphorylation of VE-cadherin. As a transmembrane protein, the intracellular domain of VE-cadherin has been shown to interact with cytoplasmic proteins called catenins that transmit the adhesion signal upon this activation. So the VE-cadherin extracellular domain is enough for early steps of cell adhesion and recognition. However, interaction of VE-cadherin with the cytoskeleton, mediated through the cytoplasmatic domain, is necessary to provide strength and cohesion to the junction.

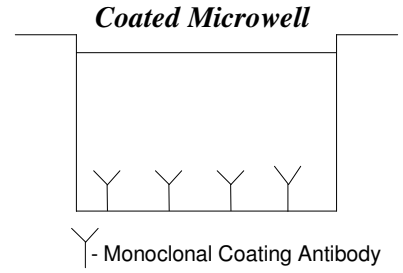
Apart from its established role in controlling the permeability of vascular endothelium, this molecule may have a similar role in perineurium, being important in the maintenance of the blood-nerve barrier. Furthermore, it functions to maintain the fibrin or collagen induced capillary tube architecture.

Specified cell adhesion molecules such as VE-cadherin are involved in the subsequent events of endothelial cell differentiation, apoptosis and angiogenesis. In immunohistochemical studies, altered VE-cadherin expression has been described for several tumors such as haemangiomas, glioblastomas and Kaposi’s sarcoma.

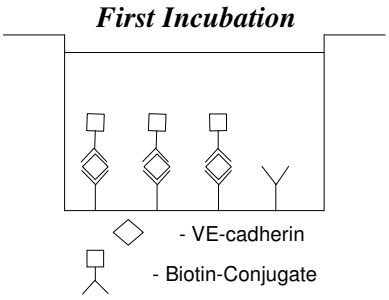
Most recently it has been shown that the initiation of endothelial apoptosis correlates with cleavage and disassembly of components of adherens junctions. The extracellular portion of these junctions is altered during apoptosis because VE-cadherin dramatically decreases on the surface of cells and an extracellular fragment of VE-cadherin can be detected. This shedding of VE-cadherin can be blocked by an inhibitor of metalloproteinases. It may be part of a concerted mechanism to disrupt structural and signaling properties of adherens junctions and may actively interrupt extracellular signals required for endothelial cell survival.

PRINCIPLE

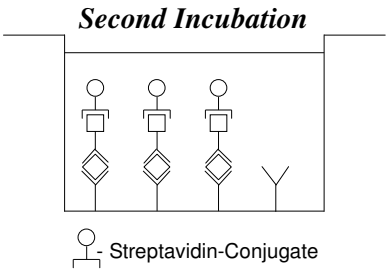
An anti-VE-cadherin coating antibody is adsorbed onto microwells.

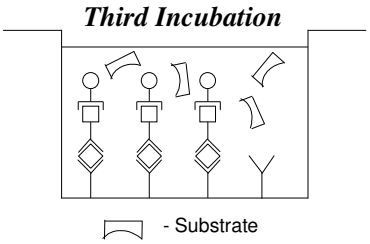


sVE-cadherin present in the sample or calibrator binds to antibodies adsorbed to the microwells; a biotin conjugated anti-VE-cadherin antibody is added and binds to sVE-cadherin captured by the first antibody.

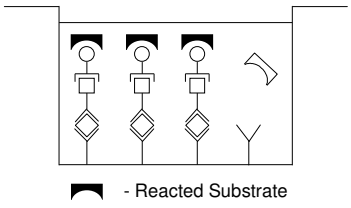


Following incubation, unbound biotin conjugated anti-VE-cadherin is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-VE-cadherin. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.





A colored product is formed in proportion to the amount of sVE-cadherin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A calibration curve is prepared from seven sVE-cadherin calibrator dilutions and sVE-cadherin sample concentration determined.



COMPONENTS

- 1 Microtiter Plate coated with Antibody to human VE-cadherin
- 1 vial (10 µL) Biotin-Conjugate anti-VE-cadherin antibody
- 1 vial (200 µL) Streptavidin-HRP
- 2 vials sVE-cadherin Calibrator, lyophilized, 20 ng/mL upon reconstitution
- 1 bottle (50 mL) Wash Buffer Concentrate, 20x (PBS with 1% Tween 20)
- 1 vial (5 mL) Assay Buffer Concentrate, 20x (PBS with 1% Tween 20 and protein stabilizer)
- 1 bottle (12 mL) Sample Diluent
- 1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)
- 1 vial (12 mL) Stop Solution (1 M Phosphoric acid)
- 1 vial (0.4 mL each) Blue-Dye, Green-Dye, Red-Dye
- 4 adhesive Plate Covers

Materials or Equipment Required but Not Provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1,000 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 Buffer (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 linear regression analysis

PROTOCOLS

Specimen Collection

Cell culture supernatants, human serum, heparin plasma or other biological samples will be suitable for use in the assay. Remove serum or plasma from the clot or red cells, respectively, 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 VE-cadherin. If samples are to be run within 24 hours, they may be stored at 4°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

For stability of samples refer to page 11. Pay attention to a possible “Hook Effect” due to high sample concentrations.

PREPARATION OF REAGENTS

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

Wash Buffer (1x)

Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1,000 mL graduated cylinder. Bring final volume to 1,000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should be 7.4.

Transfer to a clean wash bottle and store at 2-25°C. Please note that the Wash Buffer (1x) is stable for 30 days. Wash Buffer (1x) may 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

Assay Buffer (1x)
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.

Store at 4ºC. Please note that the Assay Buffer (1x) is stable for 30 days. 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

Preparation of Biotin-Conjugate
Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.
Add 90 µL of Assay Buffer (1x) to the vial containing the Biotin-Conjugate concentrate.
Make a further 1:100 dilution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

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

Store any diluted stock not needed immediately at -20°C.

Preparation of sVE-cadherin Calibrator
Reconstitute sVE-cadherin Calibrator by addition of distilled water. Reconstitution volume is stated on the label of the calibrator vial. Make sure the contents entirely dissolve by gentle swirling. The calibrator solution obtained is 20 ng/mL. After usage, the remaining calibrator cannot be stored and has to be discarded.

Preparation of Streptavidin-HRP
Please note that Streptavidin-HRP should be used within 30 minutes after dilution.
Make a 1:100 dilution of the concentrated Streptavidin-HRP solution as needed according to the following table:

Number of Strips	Stretavidin-HRP (mL)	Assay Buffer (1x) (mL)
1-6	0.06	5.94
1-12	0.12	11.88

Addition of color-giving reagents: Blue-Dye, Green-Dye, Red-Dye
In order to help our customers to avoid any mistakes in pipetting the **K-*ASSAY*®** Human sVE-cadherin ELISA, KAMIYA BIOMEDICAL COMPANY now offers a new tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colors to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the package insert.
Alternatively, the dye solutions from the stocks provided (Blue-Dye, Green-Dye, Red-Dye) can be added to the reagents according to the following guidelines:

1. Diluent: Before sample dilution add the Blue-Dye at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of Blue-Dye, proceed according to the package insert.

5 mL Diluent	20 µL Blue-Dye
12 mL Diluent	48 µL Blue-Dye
50 mL Diluent	200 µL Blue-Dye

2. Biotin-Conjugate: Before dilution of the concentrated conjugate, add the Green-Dye at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of Green-Dye according to the package insert, preparation of Biotin-conjugate.

3 mL Assay Buffer (1x)	30 µL Green-Dye
6 mL Assay Buffer (1x)	60 µL Green-Dye

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP; add the Red-Dye at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of Red-Dye according to the package insert, preparation of Streptavidin-HRP.

6 mL Assay Buffer (1x)	24 µL Red-Dye
12 mL Assay Buffer (1x)	48 µL Red-Dye

TEST PROTOCOL

a. Mix all reagents thoroughly without foaming before use.

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 calibrators. Each sample, calibrator, blank, and optional control sample should be assayed in duplicate. Remove extra microwell strips coated with antibody to human VE-cadherin from holder and store in foil bag with the desiccant provided at 4°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, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µL of Sample Diluent in duplicate to all calibrator wells. Prepare calibrator dilutions by pipetting 100 µL of reconstituted (refer to preparation of reagents) sVE-cadherin Calibrator, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µL to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of sVE-cadherin Calibrator dilutions ranging from 10.00 to 0.16 ng/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of sVE-cadherin Calibrator dilutions:

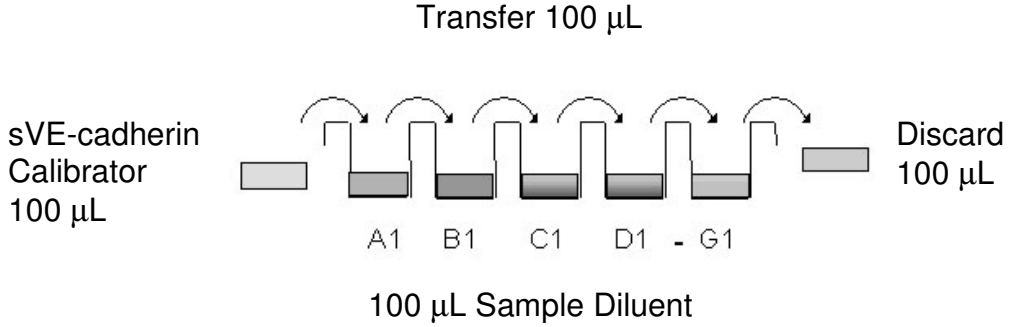


Figure 2. Diagram depicting an example of the arrangement of blanks, calibrators and samples in the microwell strips:

	1	2	3	4
A	Calibrator 1 (10 ng/mL)	Calibrator 1 (10 ng/mL)	Sample 1	Sample 1
B	Calibrator 2 (5 ng/mL)	Calibrator 2 (5 ng/mL)	Sample 2	Sample 2
C	Calibrator 3 (2.5 ng/mL)	Calibrator 3 (2.5 ng/mL)	Sample 3	Sample 3
D	Calibrator 4 (1.25 ng/mL)	Calibrator 4 (1.25 ng/mL)	Sample 4	Sample 4
E	Calibrator 5 (0.63 ng/mL)	Calibrator 5 (0.63 ng/mL)	Sample 5	Sample 5
F	Calibrator 6 (0.31 ng/mL)	Calibrator 6 (0.31 ng/mL)	Sample 6	Sample 6
G	Calibrator 7 (0.16 ng/mL)	Calibrator 7 (0.16 ng/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µL of Sample Diluent in duplicate to the blank wells.

f. Add 80 µL of Sample Diluent to the sample wells.

g. Add 20 µL of each Sample, in duplicate, to the designated wells.

h. Prepare Biotin-Conjugate (refer to preparation of reagents).

i. Add 50 µL of diluted Biotin-Conjugate to all wells, including the blank wells.

j. Cover with a Plate Cover and incubate at room temperature (RT) (18-25°C) for 2 hours on a microplate shaker set at 100 rpm.

k. Prepare Streptavidin-HRP (refer to preparation of reagents).

l. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to step ‘c’ of the test protocol. Proceed immediately to the next step.

m. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.

n. Cover with a Plate Cover and incubate at RT for 1 hour on a microplate shaker at 100 rpm.

o. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to step ‘c’ of the test protocol. Proceed immediately to the next step.

p. Pipette 100 µL of TMB Substrate Solution to all wells, including the blank wells.

r. Incubate the microwell strips at RT for about 10 minutes. Avoid direct exposure to intense light.

The color development on the plate should be monitored and substrate reaction stopped (see step ‘s’ of the 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 calibrator 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 calibrator 1 has reached an OD of 0.6-0.65.

r. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well, including the blank wells. 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 4℃ in the dark.

t. Read absorbance of each microwell on a spectrophotometer 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 sVE-cadherin calibrators.

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

CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate calibrators and samples. Duplicates should be within 20 per cent of the mean.

- Create a calibration curve by plotting the mean absorbance for each calibrator concentration on the ordinate against the sVE-cadherin 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 sVE-cadherin for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the calibration curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding sVE-cadherin concentration.

  For samples which have been diluted 1:5 according to the instructions given in this manual, the concentration read from the calibration curve must be multiplied by the dilution factor (x5).

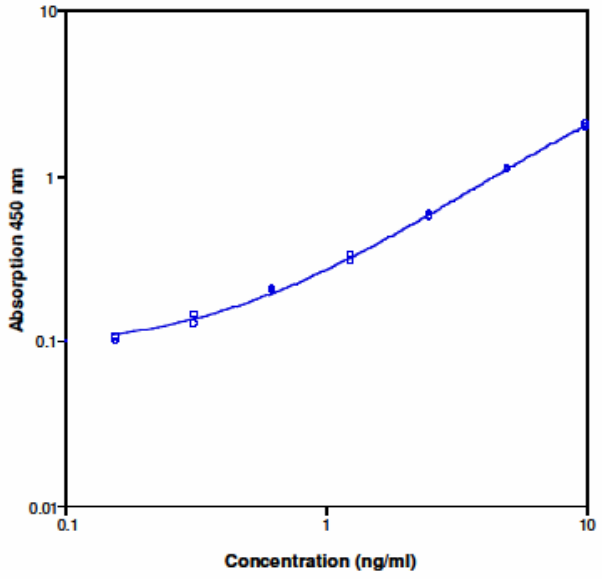
  Note: Calculation of samples with a concentration exceeding calibrator 1 may result in incorrect, low sVE-cadherin levels ("Hook Effect"). Such samples require further external predilution according to expected human sVE-cadherin values with Sample Diluent in order to precisely quantitate the actual human sVE-cadherin levels.

  It is suggested that each testing facility establishes a control sample of known sVE-cadherin concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative calibration curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a calibration curve for each group of microwell strips assayed.

EXPECTED RESULTS

Figure 3. Representative calibration curve for the **K-*ASSAY*®** Human sVE-cadherin ELISA. sVE-cadherin was diluted in serial two-fold steps in Sample Diluent. Do not use this calibration curve to derive test results. A calibration curve must be run for each group of microwell strips assayed.



Typical data using the sVE-cadherin ELISA:
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Calibrator	sVE-cadherin Concentration (ng/mL)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	10	1.954	1.983	2.0
	10	2.011		
2	5	1.101	1.083	1.6
	5	1.072		
3	2.5	0.582	0.568	3.7
	2.5	0.552		
4	1.25	0.329	0.315	6.8
	1.25	0.299		
5	0.63	0.205	0.204	1.4
	0.63	0.201		
6	0.31	0.143	0.135	9.5
	0.31	0.125		
7	0.16	0.106	0.103	4.8
	0.16	0.099		
Blank	0	0.080	0.083	
	0	0.085		

The OD values of the calibration curve may vary according to the conditions of assay performance (i.e. 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.

LIMITATIONS

- Since exact conditions may vary from assay to assay, a calibration curve must be established for every run.
- Bacterial or fungal contamination of either 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. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

PERFORMANCE CHARACTERISTICS

Sensitivity

The limit of detection of sVE-cadherin defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.1 ng/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sVE-cadherin. Two calibration curves were run on

each plate. Data below show the mean sVE-cadherin concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 4.1%.

Sample	Experiment	sVE-cadherin Concentration (ng/mL)	Coefficient of Variation (%)
1	1	14.7	10.4
	2	14.0	11.1
	3	12.3	6.3
2	1	27.4	2.4
	2	33.0	4.5
	3	33.5	4.8
3	1	49.4	2.6
	2	51.5	0.7
	3	58.8	3.2
4	1	15.3	2.9
	2	16.2	4.1
	3	16.8	2.7
5	1	17.0	2.6
	2	18.1	2.1
	3	18.5	6.8
6	1	15.4	1.9
	2	16.9	2.7
	3	17.1	4.6
7	1	16.3	7.1
	2	16.6	0.9
	3	18.0	2.7
8	1	15.6	5.1
	2	16.4	3.7
	3	18.5	2.9

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sVE-cadherin. Two calibration curves were run on each plate. Data below show the mean sVE-cadherin concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 7.2%.

Sample	sVE-cadherin Concentration (ng/mL)	Coefficient of Variation (%)
1	13.7	9.2
2	31.3	10.7
3	53.2	9.2
4	16.1	4.8
5	17.9	4.1
6	16.4	5.6
7	17.0	5.4
8	16.8	8.9

Spike Recovery

The spike recovery was evaluated by spiking four levels of sVE-cadherin into 4 different pooled normal human serum samples. Recoveries were determined in 3 independent experiments with 4 replicates each. The amount of endogenous sVE-cadherin in unspiked serum was subtracted from the spike values. Recoveries ranged from 61% to 109% with an overall mean recovery of 82%.

Dilution Parallelism

Four serum samples with different levels of sVE-cadherin were assayed at serial two-fold dilutions with 4 replicates each. In the table below the percent recovery of expected values is listed. Recoveries ranged from 100% to 122% with an overall mean recovery of 113%.

Sample	Dilution	sVE-cadherin Concentration (ng/mL) Expected Value	Observed Value	% Recovery of Exp. Value
1	1:5	--	19.3	--
	1:10	9.7	11.3	116
	1:20	5.7	6.1	107
	1:40	3.1	3.7	119
2	1:5	--	17.6	--
	1:10	8.8	10.1	115
	1:20	5.01	5.2	103
	1:40	2.6	3.0	115
3	1:5	--	16.3	--
	1:10	8.2	9.9	120
	1:20	5.0	6.1	122
	1:40	3.0	3.5	117
4	1:5	--	25.9	--
	1:10	12.9	13.0	100
	1:20	6.5	6.8	104
	1:40	3.4	3.8	114

SAMPLE STABILITY

Freeze-Thaw Stability

Aliquots of serum and cell culture supernatant samples (unspiked or spiked) were stored frozen at -20℃ and thawed 5 times, and sVE-cadherin levels determined. There was no significant loss of sVE-cadherin immunoreactivity detected by freezing and thawing.

Storage Stability

Aliquots of serum and cell culture supernatant samples (spiked or unspiked) were stored at -20℃, 4℃, RT and at 37℃, and the sVE-cadherin level determined after 24 hours. There was no significant loss of sVE-cadherin immunoreactivity during storage under above conditions.

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a sVE-cadherin positive serum. There was no detectable cross reactivity.

STORAGE

Store Calibrator and Biotin-Conjugate at -20℃. Store other kit reagents at 4℃. Immediately after use remaining reagents should be returned to cold storage as indicated. Expiration date of the kit and reagents is stated on labels.

The expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

WARNINGS AND PRECAUTIONS

- 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 pipette 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 specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens which 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 acids will inactivate the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at RT prior to use.
- Decontaminate and dispose specimens 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.
- All chemicals 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.

REAGENT PREPARATION SUMMARY

A. 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

B. 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

C. Biotin-Conjugate Pre-dilution: Add 90 µL Assay Buffer (1x) to vial containing Biotin-Conjugate concentrate. Mix. Make a further 1:100 dilution according to the table:

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

D. sVE-cadherin Calibrator Add the volume of distilled water as stated on label to each vial of lyophilized sVE-cadherin Calibrator as needed.

E. **Streptavidin-HRP** Make a 1:100 dilution of Streptavidin-HRP in Assay Buffer (1x):

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1-6	0.06	5.94
1-12	0.12	11.88

TEST PROTOCOL SUMMARY

- Determine the number of microwell strips required
- Wash microwell strips twice with Wash Buffer
- Add 100 µL Sample Diluent, in duplicate, to all calibrator wells

- Pipette 100 µL reconstituted sVE-cadherin Calibrator into the first wells and create calibrator dilutions ranging from 10 to 0.16 ng/mL by transferring 100 µL from well to well. Discard 100 µL from the last wells
- Add 100 µL Sample Diluent, in duplicate, to the blank wells
- Add 80 µL Sample Diluent to the sample wells
- Add 20 µL Sample, in duplicate, to designated wells
- Prepare Biotin-Conjugate
- Add 50 µL of diluted Biotin-Conjugate to all wells
- Cover microwell strips and incubate 2 hours at RT
- Prepare Streptavidin-HRP
- Empty and wash microwell strips 3 times with Wash Buffer
- Add 100 µL of diluted Streptavidin-HRP to all wells
- Cover microwell strips and incubate 1 hour at RT
- Empty and wash microwell strips 3 times with Wash Buffer
- Add 100 µL of TMB Substrate Solution to all wells including blank wells
- Incubate the microwell strips for about 10 minutes at RT
- Add 100 µL Stop Solution to all wells including blank wells
- Blank microwell reader and measure color intensity at 450 nm

Note: For samples which have been diluted according to the instructions given in this manual 1:5, the concentration read from the calibration curve must be multiplied by the dilution factor (x5).

FOR RESEARCH USE ONLY

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