Product Manual

Human VE-cadherin ELISA Kit

ALX-850-059A

Enzyme-linked Immunosorbent Assay for quantitative detection of human VE-cadherin.

For research use only.
Not for diagnostic or therapeutic procedures.

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1 Intended Use

This immunoassay kit allows for the *in vitro* quantitative determination of human cadherin-5 concentrations in serum, plasma, urine, tissue homogenates, cell culture supernatants, and other biological fluids. **The human VE-cadherin ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

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

VE-cadherin belongs to the adhesion molecules responsible for cellular interactions. The vascular endothelial cadherin (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. 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. VE-cadherin is a transmembrane protein, the intracellular domain 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 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. It furthermore 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. 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.

For literature update refer to **www.enzolifesciences.com**

3 Principles of the Test

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

Figure 1

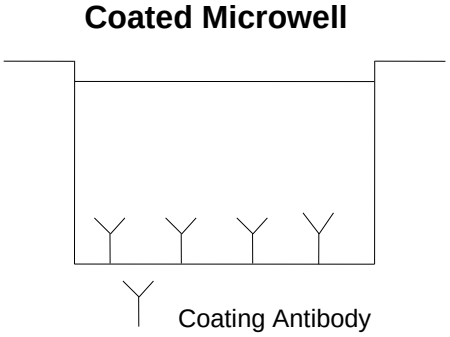


Figure 2

Human VE-cadherin present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human VE-cadherin antibody is added and binds to human VE-cadherin captured by the first antibody.

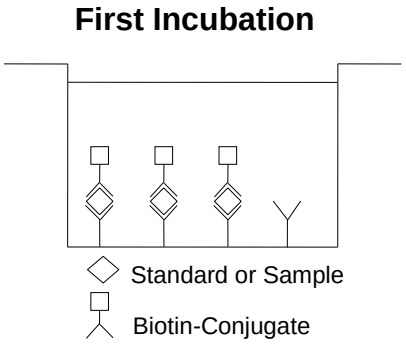


Figure 3

Following incubation unbound biotin-conjugated anti-human VE-cadherin antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human VE-cadherin antibody.

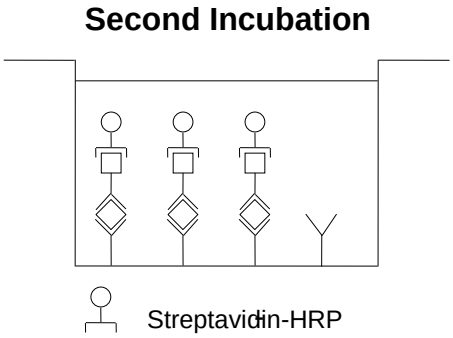
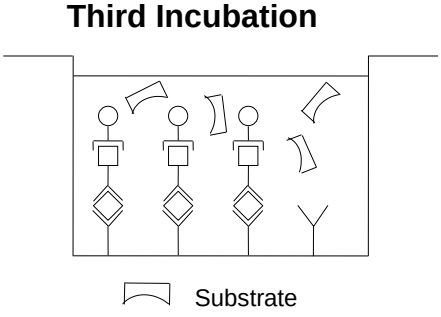


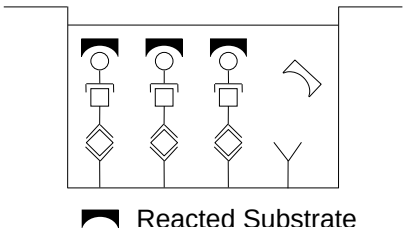
Figure 4

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



A coloured product is formed in proportion to the amount of human VE-cadherin 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 7 human VE-cadherin standard dilutions and human VE-cadherin sample concentration determined.

Figure 5



4 Reagents Provided

Reagent	Quantity
Assay Plate	1
Standard	2
Sample Diluent	1 x 20 mL
Assay Diluent A	1 x 10 mL
Assay Diluent B	1 x 10 mL
Detection Reagent A	1 x 120 µL
Detection Reagent B	1 x 120 µL
Wash Buffer (25X)	1 x 30 mL
Substrate	1 x 10 mL
Stop Solution	1 x 10 mL
Plate Sealer	5

5 Storage Instructions – ELISA Kit

The **Assay Plate, Standard, Detection Reagent A, and Detection Reagent B** should be stored at -20°C upon being received. **Substrate should always be stored at +4°C.** Other reagents are kept according to the labels on the vials. For long term storage, store the entire kit at –20°C.

6 Specimen Collection and Storage Instructions

Serum – Use a serum separator tube (SST), and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma – Collect plasma using EDTA or heparin as an anti-coagulant. Centrifuge samples for 15 minutes at 1000 x g at 2°C – 8°C within 30 minutes of collection. Store samples at -20°C or -80°C, and avoid freeze-thaw cycles.

Urine – Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge toi remove particulate matter, assay immediately or aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates – The preparation of tissue homogenates with vary depending on tissue type. For this assay, tissue was rinsed with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS, and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at -20°C.

Cell Culture Supernates and Other Biological Fluids – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

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 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. 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 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 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 specimens and all potentially contaminated materials as 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.

a. 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.

i. Wash Buffer

If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer concentrate into deionized water to prepare 750 mL of Wash Buffer.

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.
Mix gently to avoid foaming.

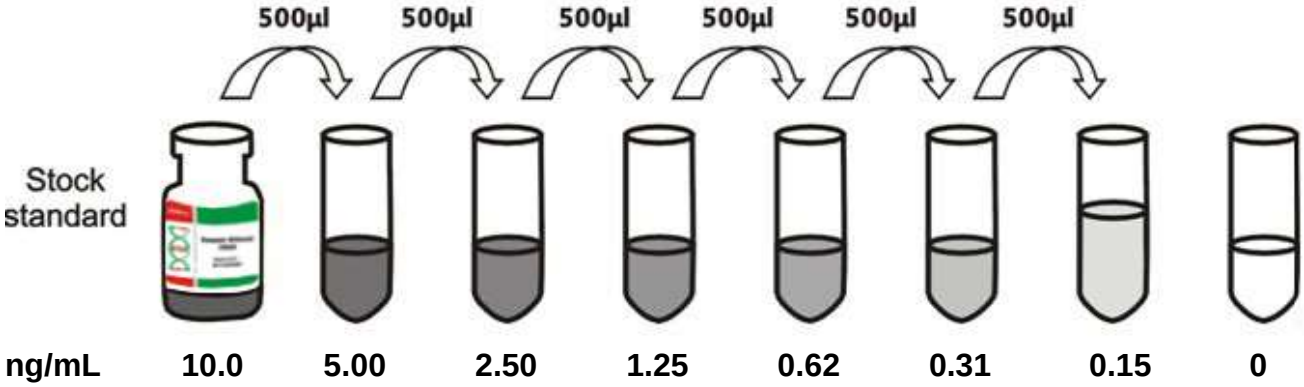
Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

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

ii. Human VE-cadherin Standard

Reconstitute **human VE-cadherin standard** with 1/0 mL of Sample Diluent. This reconstitution process produces a stock solution of 10.0 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. We do not recommend making serial dilutions directly in the wells. The undiluted standard serves as the high standard (10.0 ng/mL). The Sample Diluent is the zero standard (0 ng/mL).



iii. Detection Reagent A and B

Dilute to the working concentration using **Assay Diluent A and B (1:100), respectively.**

Test Protocol

Allow all reagents to come room temperature (please do not dissolve the reagents directly at 37°C). **All reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Keep the appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be reseals and stored at 4°C until the kit's expiry date. Prepare all reagents, working standards, and samples as directed previously. Please predict the concentration before assaying; if expected values are not within the range of the standard curve, dilutions may be required.

1. Add 100 µL of **Standard**, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37℃.

2. Remove the liquid of each well, don't wash. Add 100 µL of **Detection Reagent A** working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37℃. **Detection Reagent A** working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µL) using a squirt bottle, multi-channel pipette, 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.

4. Add 100 µL of **Detection Reagent B** working solution to each well. Cover with a new Plate sealer. Incubate for 1 hour at 37℃.

5. Repeat the aspiration/wash as in step 4.

6. Add 90 µL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate within 15-30 minutes at 37℃. Protect from light.

7. Add 50 µL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Note:

1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.

2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards Detection Reagent A and B can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.

3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay.

4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.

5. 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.

6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

7. Duplication of all standards and specimens, although not required, is recommended.

8. Substrate Solution is easily contaminated. Please protect it from light.

b. Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 1.3. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Specificity: This assay recognized recombinant and natural human VE-cadherin.

Sensitivity: The minimum detectable dose of human VE-cadherin is typically 0.051 ng/mL. The sensitivity of this assay, or LOD, was defined as the lowest protein concentration that could be differentiated from zero.

Detection Range: 0.156 ng/mL – 10 ng/mL

Notes:

Notes:

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