PRODUCT INFORMATION AND MANUAL

Monkey VE-cadherin ELISA

IB49726
and
IB49726-10

Enzyme-linked immunosorbent assay for

detection of monkey VE-cadherin.

For research use only.

Not for diagnostic or therapeutic procedures.

Monkey VE-cadherin
IB49726
IB49726-10

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1 Intended Use

The monkey VE-cadherin ELISA is an enzyme-linked immunosorbent assay for the detection of monkey VE-cadherin. **The monkey 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.

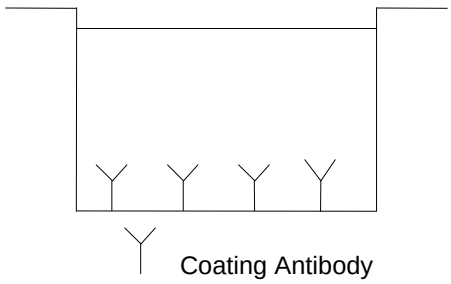
For literature update refer to **www.ibl-america.com**

3 Principles of the Test

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

Figure 1

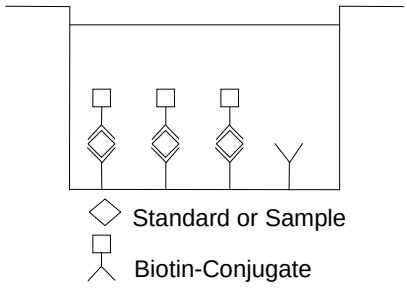
Coated Microwell



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

Figure 2

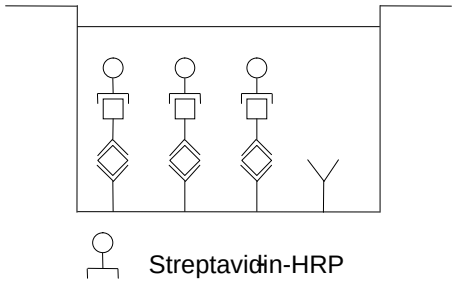
First Incubation



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

Figure 3

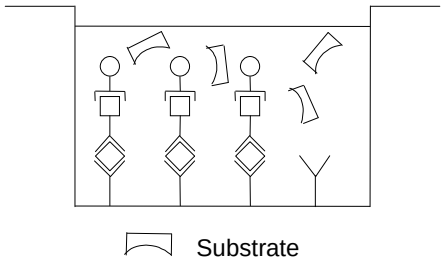
Second Incubation



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

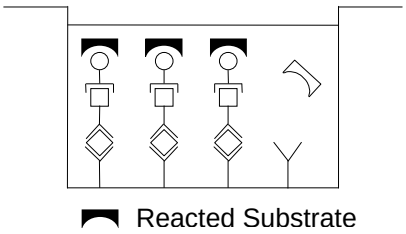
Figure 4

Third Incubation



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

Figure 5



4 Reagents Provided

4.1 Reagents for monkey VE-cadherin ELISA IB49726 (96 tests)

1 aluminium pouch with a **Microwell Plate coated** with polyclonal antibody to monkey VE-cadherin

1 vial (10 µl) **Biotin-Conjugate** anti-monkey VE-cadherin polyclonal antibody

1 vial (200 µl) **Streptavidin-HRP**

2 vials monkey VE-cadherin **Standard** lyophilized, 25 U/ml upon reconstitution

1 vial (12 ml) **Sample Diluent**

1 vial (5 ml) **Assay Buffer Concentrate** 20x
(PBS with 1% Tween 20 and 10% BSA)

1 bottle (50 ml) **Wash Buffer Concentrate** 20x
(PBS with 1% Tween 20)

1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml) **Blue-Dye**

1 vial (0.4 ml) **Green-Dye**

1 vial (0.4 ml) **Red-Dye**

4 **Adhesive Films**

4.2 Reagents for monkey VE-cadherin ELISA IB49726-10 (10x96 tests)

10 aluminium pouches with a **Microwell Plate coated** with polyclonal antibody to monkey VE-cadherin

10 vials (10 µl) **Biotin-Conjugate** anti-monkey VE-cadherin polyclonal antibody

10 vials (200 µl) **Streptavidin-HRP**

10 monkey VE-cadherin **Standard** lyophilized, 25 U/ml upon reconstitution

10 vials (12 ml) **Sample Diluent**

2 vials (5 ml) **Assay Buffer Concentrate**
20x (PBS with 1% Tween 20 and 10% BSA)

4 bottles (50 ml) **Wash Buffer Concentrate**
20x (PBS with 1% Tween 20)

10 vials (15 ml) **Substrate Solution** (tetramethyl-benzidine)

10 vials (15 ml) **Stop Solution** (1M Phosphoric acid)

6 vials (0.4 ml) **Blue-Dye**

6 vials (0.4 ml) **Green-Dye**

6 vials (0.4 ml) **Red-Dye**

20 **Adhesive Films**

5 Storage Instructions – ELISA Kit

Store standard and Biotin-Conjugate at -20°C. Store other kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (standard and Biotin-Conjugate at -20°C, other kit reagents at 2° to 8°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 Specimen Collection and Storage Instructions

Cell culture supernatant, serum and plasma (heparinized) 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 monkey VE-cadherin. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13.5).
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 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.

9 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.

9.1 Wash Buffer (1x)

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. The pH of the final solution should adjust to 7.4.

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

9.2 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 2° to 8°C. 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

9.3 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 of the concentrated **Biotin-Conjugate** solution 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**.

9.4 Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

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

9.5 Monkey VE-cadherin Standard

Reconstitute **monkey VE-cadherin 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 = 25.00 U/ml).
Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

After usage remaining standard cannot be stored and has to be discarded.

Standard dilutions can be prepared directly on the microwell plate (see 10.c) or alternatively in tubes (see 9.5.1).

9.5.1 External Standard Dilution

Label 7 tubes, one for each standard point.

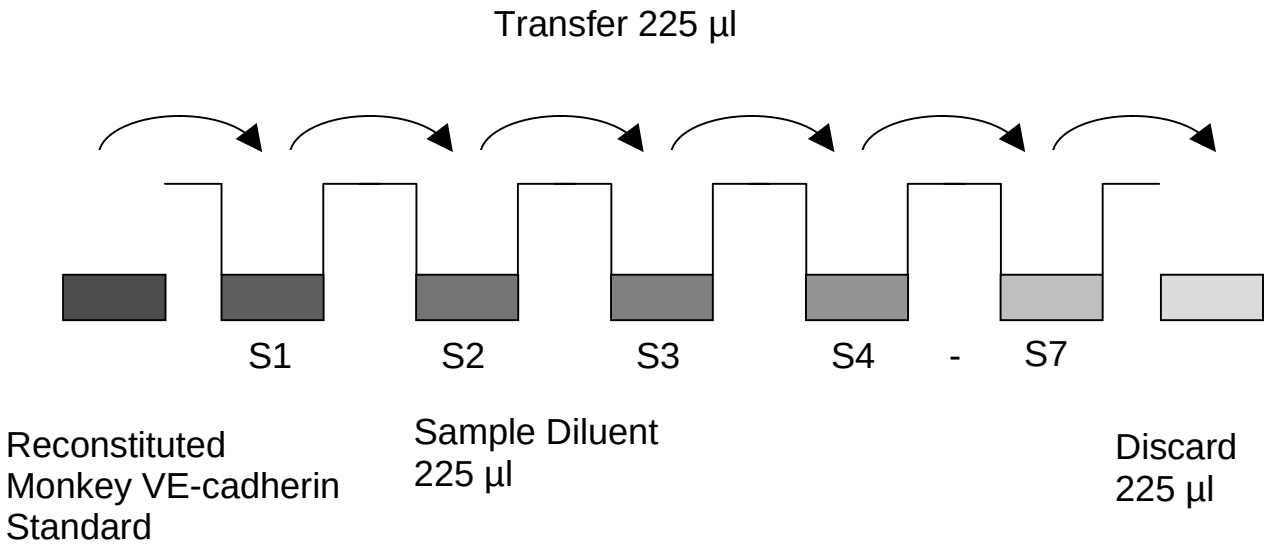
S1, S2, S3, S4, S5, S6, S7

Then prepare 1:2 serial dilutions for the standard curve as follows:
Pipette 225 µl of Sample Diluent into each tube.

Pipette 225 µl of reconstituted standard (concentration = 25.00 U/ml) into the first tube, labelled S1, and mix (concentration of standard 1 = 12.50 U/ml).
Pipette 225 µl of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.
Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6).

Sample Diluent serves as blank.

Figure 6



9.6 Addition of Colour-giving Reagents: Blue-Dye, Green-Dye, Red-Dye

In order to help our customers to avoid any mistakes in pipetting the IBL-America ELISAs, IBL-America offers a tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours 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 instruction booklet.

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 standard and 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 instruction booklet.

5 ml Sample Diluent	20 µl ***Blue-Dye***
12 ml Sample Diluent	48 µl ***Blue-Dye***
50 ml Sample Diluent	200 µl ***Blue-Dye***

2. Biotin-Conjugate: Before dilution of the concentrated Biotin-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 instruction booklet: Preparation of Biotin-Conjugate.

3 ml Assay Buffer (1x)	30 µl ***Green-Dye***
6 ml Assay Buffer (1x)	60 µl ***Green-Dye***
12 ml Assay Buffer (1x)	120 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 instruction booklet: Preparation of Streptavidin-HRP.

6 ml Assay Buffer (1x)	24 µl ***Red-Dye***
12 ml Assay Buffer (1x)	48 µl ***Red-Dye***

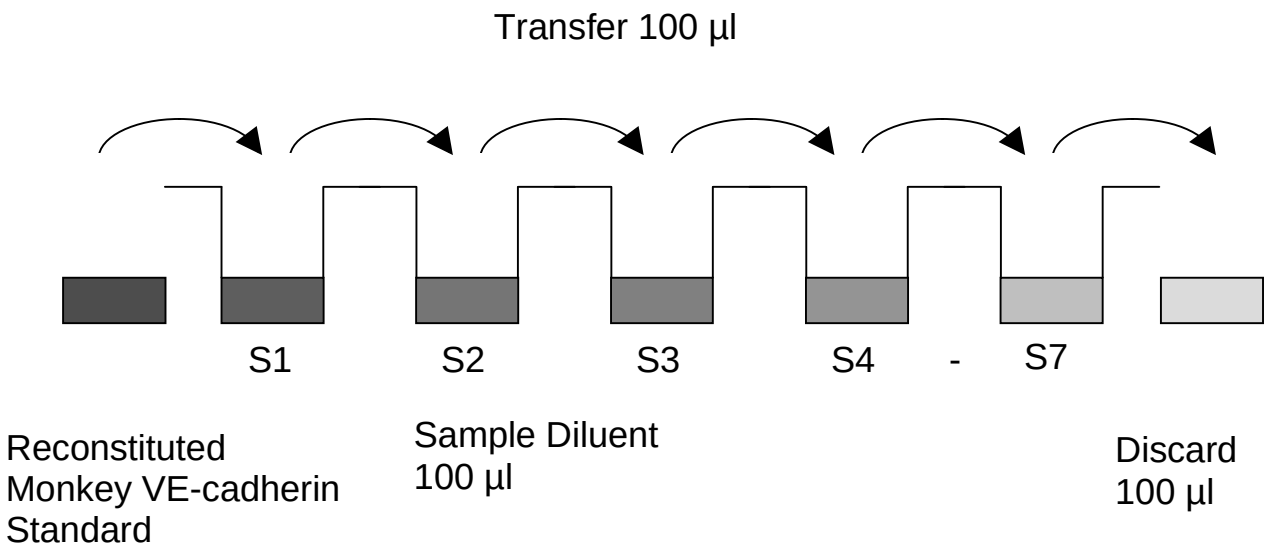
10 Test Protocol

a. 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 sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

b. 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**.

c. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 9.5.1):
Add 100 µl of Sample Diluent in duplicate to all **standard wells**. Pipette 100 µl of prepared **standard** (see Preparation of Standard 9.5, concentration = 25.00 U/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 = 12.50 U/ml), and transfer 100 µl to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of monkey VE-cadherin standard dilutions ranging from 12.50 to 0.19 U/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 7


Transfer 100 µl
S1
S2
S3
S4
-
S7
Reconstituted
Monkey VE-cadherin
Standard
Sample Diluent
100 µl
Discard
100 µl

In case of an **external standard dilution** (see 9.5.1), pipette 100 µl of these standard dilutions (S1 - S7) 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 (12.50 U/ml)	Standard 1 (12.50 U/ml)	Sample 1	Sample 1
B	Standard 2 (6.25 U/ml)	Standard 2 (6.25 U/ml)	Sample 2	Sample 2
C	Standard 3 (3.13 U/ml)	Standard 3 (3.13 U/ml)	Sample 3	Sample 3
D	Standard 4 (1.56 U/ml)	Standard 4 (1.56 U/ml)	Sample 4	Sample 4
E	Standard 5 (0.78 U/ml)	Standard 5 (0.78 U/ml)	Sample 5	Sample 5
F	Standard 6 (0.39 U/ml)	Standard 6 (0.39 U/ml)	Sample 6	Sample 6
G	Standard 7 (0.19 U/ml)	Standard 7 (0.19 U/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 100 µl of **Sample Diluent** in duplicate to the **blank wells**.

e. Add 80 µl of **Sample Diluent** to the **sample wells**.

f. Add 20 µl of each **sample** in duplicate to the **sample wells**.

g. Prepare **Biotin-Conjugate** (see Preparation of Biotin-Conjugate 9.3).

h. Add 50 µl of **Biotin-Conjugate y** to all 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 set at 100 rpm.

j. Prepare **Streptavidin-HRP** (refer to Preparation of Streptavidin-HRP 9.4).

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

l. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

m. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 100 rpm.

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

o. Pipette 100 µl of **TMB Substrate Solution** to all wells.

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

The colour 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 colour 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 colour. Alternatively the colour 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.

q. 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.

r. 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 per cent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the monkey VE-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 monkey VE-cadherin 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 monkey VE-cadherin concentration.
- **If instructions in this protocol have been followed samples have been diluted 1:5 (20 µl sample + 80 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 5).**
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low monkey VE-cadherin levels. Such samples require further external predilution according to expected monkey VE-cadherin values with Sample Diluent in order to precisely measure the actual monkey VE-cadherin level.**
- It is suggested that each testing facility establishes a control sample of known monkey VE-cadherin 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 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 8

Representative standard curve for monkey VE-cadherin ELISA. Monkey VE-cadherin was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

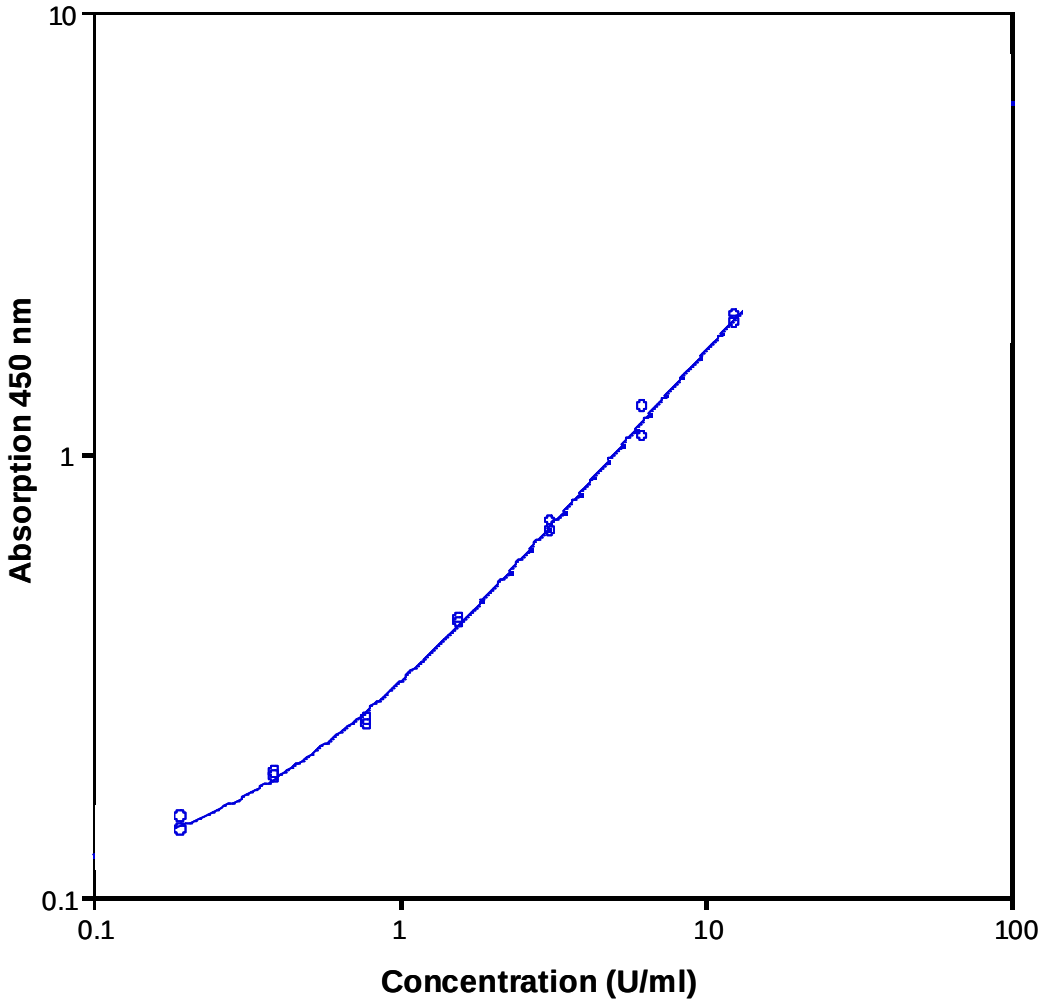


Table 2
Typical data using the monkey VE-cadherin ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Monkey VE-cadherin Concentration (U/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	12.50	2.055	2.014	2.9
	12.50	1.974		
2	6.25	1.095	1.184	10.6
	6.25	1.273		
3	3.13	0.703	0.685	3.6
	3.13	0.668		
4	1.56	0.415	0.420	1.7
	1.56	0.425		
5	0.78	0.245	0.248	1.8
	0.78	0.251		
6	0.39	0.191	0.189	1.9
	0.39	0.186		
7	0.19	0.141	0.146	4.8
	0.19	0.151		
Blank	0	0.087	0.086	1.6
	0	0.085		

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 colour intensity. Values measured are still valid.

12 Limitations

- Since 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.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of monkey VE-cadherin 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 0.10 U/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 samples containing different concentrations of monkey VE-cadherin. 2 standard curves were run on each plate. The overall intra-assay coefficient of variation has been calculated to be <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 samples containing different concentrations of monkey VE-cadherin. 2 standard curves were run on each plate. The overall inter-assay coefficient of variation has been calculated to be <10%.

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of monkey VE-cadherin into samples (serum matrix). Recoveries were determined in 3 independent experiments with 4 replicates each.
The amount of endogenous monkey VE-cadherin in unspiked sample was subtracted from the spike values.
The overall mean recovery was 80%.

13.4 Dilution Parallelism

4 samples with different levels of monkey VE-cadherin were analysed at serial 2 fold dilutions with 4 replicates each.
The overall mean recovery was 109%.

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

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

13.5.2 Storage Stability

Aliquots of samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the monkey VE-cadherin level determined after 24 h. There was no significant loss of monkey VE-cadherin immunoreactivity detected during storage under above conditions.

14 Ordering Information

For literature update refer to **www.ibl-america.com**

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Cat.No. IB49726 monkey VE-cadherin ELISA

Cat.No. IB49726-10 monkey VE-cadherin ELISA

15 Reagent Preparation Summary

15.1 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

15.2 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

15.3 Biotin-Conjugate

Add 90µl Assay Buffer (1x) to the tube containing the **Biotin-Conjugate** concentrate. Make a further 1:100 dilution of Biotin-Conjugate in Assay Buffer (1x):

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

15.4 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

15.5 Monkey VE-cadherin Standard

Reconstitute lyophilized **monkey VE-cadherin standard** with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

16 Test Protocol Summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µl Sample Diluent, 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 9.5.1): Pipette 100 µl of these standard dilutions in the microwell strips.
4. Add 100 µl Sample Diluent, in duplicate, to the blank wells.
5. Add 80 µl Sample Diluent to sample wells.
6. Add 20 µl sample in duplicate, to designated sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50 µl Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 3 times with Wash Buffer.
12. Add 100 µl diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C).
14. Empty and wash microwell strips 3 times with Wash Buffer.
15. Add 100 µl of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
17. Add 100 µl Stop Solution to all wells.
18. Blank microwell reader and measure colour intensity at 450 nm.

Note: If instructions in this protocol have been followed samples have been diluted 1:5 (20 µl sample + 80 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 5).