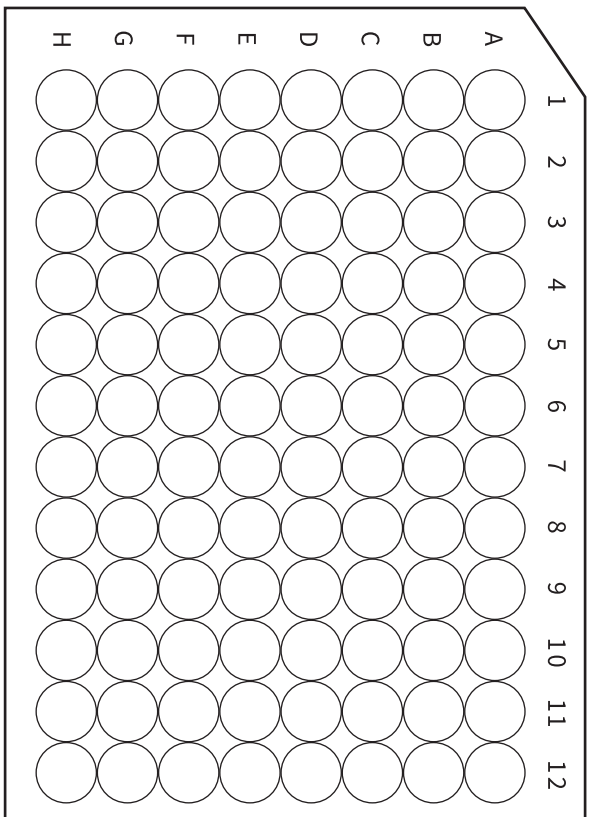

DESCRIPTION

Human Vitronectin/VTN Immunoassay

Catalog Number: SEKH-0342

For the quantitative determination of human Vitronectin concentrations in cellculture supernates,serum, and plasma.

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

MANUFACTURED AND DISTRIBUTED BY:

Country | Company: China | Beijing Solarbio Science & Technology Co.,Ltd
Address:NO.85A, Liandong U Valley, Tongzhou District, Beijing, P.R.China.
Tel: 86-10-56371241 Fax: 86-10-56371282 E-mail: service@solarbio.com

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of VTN in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	93	97
	Range(%)	83-104	82-113
1:4	Average% of Expected	95	98
	Range(%)	86-104	83-112

REFERENCES

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 15pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant human VTN. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.
REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of VTN spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of VTN in two matrices

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	91	82-102
Cell culture supernatants	94	85-103

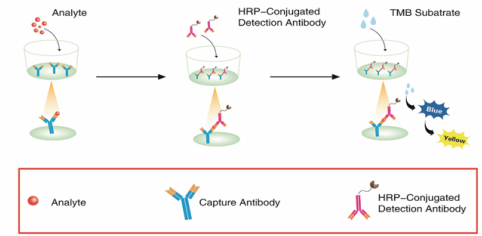
BACKGROUND

Vitronectin, also known as VTN, is a member of the pexin family. It is an abundant glycoprotein found in serum the extracellular matrix and promotes cell adhesion and spreading. Vitronectin is a secreted protein and exists in either a single chain form or a cleaved, two chain form held together by a disulfide bond. Vitronectin is a plasma glycoprotein implicated as a regulator of diverse physiological process, including blood coagulation, fibrinolysis, pericellular proteolysis, complement dependent immune responses, and cell attachment and spreading. Because of its ability to bind platelet glycoproteins and mediate platelet adhesion and aggregation at sites of vascular injury, vitronectin has become an important mediator in the pathogenesis of coronary atherosclerosis. As a multifunctional protein with a multiple binding domain, Vitronectin interacts with a variety of plasma and cell proteins. Vitronectin binds multiple ligands, including the soluble vitronectin receptor. It may be an independent predictor of adverse cardiovascular outcomes following acute stenting. Accordingly, Vitronectin is suggested to be involved in hemostasis, cell migration, as well as tumor malignancy.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VTN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any VTN present is captured by the coated antibody after incubation. Following extensive washing, a Detection antibody specific for VTN is added to detect the captured VTN protein in sample, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

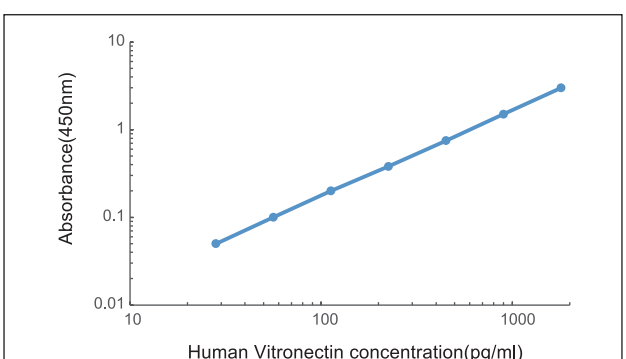
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the VTN ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.049	0.048	-------
28.13	0.182	0.195	0.188	0.140
56.3	0.264	0.284	0.274	0.226
113	0.393	0.423	0.408	0.359
225	0.624	0.671	0.647	0.599
450	1.013	1.090	1.051	1.003
900	1.649	1.775	1.712	1.663
1800	2.686	2.892	2.789	2.741



Representative standard curve for VTN ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, incubate 120 minutes, at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Detection Antibody to each well, incubate 60 minutes, at room temperature(25±2℃).

⇩ Aspirate and wash 5times

Add 100μl Substrate solution to each well, incubate 5-30 minutes, at room temperature(25±2℃).

⇩

Add 50μl Stop solution to each well. Read at 450nm within 30 minutes.

Note:oscillatory reaction at room temperature 400r or so

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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 y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the VTN concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,1800 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Detection Antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C **for six months
Detection Antibody Diluent- 16ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

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

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.
6.Human VTN controls (optional; available from Solarbio).

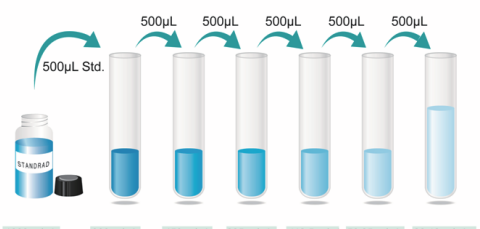
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately oraliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃.Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard\Sample**- Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 1800 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µL of Standard/Sample Diluent into 900pg/ml tube and the remaining tubes. Use the stock solution of 1800pg/mL to produce a 2-folddilution series (below).Mix each tube thoroughly and change pipette tips between each transfer. The1800pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of Detection Antibody**: Make a 1:100 dilution of the concentrated Detection Antibody with the Detection Antibody. Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**



Preparation of VTN standard dilutions