IMMBIOMED
ImmBioMed GmbH & Co. KG
Bergstr. 85, DE-64319 Pfungstadt, Germany
Phone: +49 6157 9799 685 Fax: +49 6157 86443

IMUBIND® Vitronectin ELISA

Product no. ADG803

Storage: 2 – 8°C **For Research Use Only!**

PRODUCT INSERT ***ENGLISH***

INTENDED USE

The IMUBIND® Vitronectin ELISA is an enzyme-linked immunosorbent assay for the quantitative determination of total Vitronectin in human plasma or serum or in any fluid where Vitronectin might be present.

EXPLANATION OF THE TEST

Human Vitronectin is a major plasma glycoprotein that exhibits multiple activities and functions as a cell adhesion molecule and regulator of coagulation. It contains the amino acid structural motif Arg-Gly-Asp (RGD), which is involved in cell attachment. Human Vitronectin circulates as a single-chain moiety of 75 kDa and a two-chain moiety of 65 kDa and 10 kDa.

Vitronectin belongs to the group of structurally and functionally homologous adhesive proteins (fibrinogen, fibronectin, Von Willebrand factor) that interact with platelets and the vessel wall in the early stages of blood clotting. When coated on surfaces, very low concentrations of Vitronectin promote endothelial cell attachment and induce spreading and migration of cells in a time- and concentration-dependent fashion.

Reduced Vitronectin plasma levels (up to 50 %) have been found in several patients suffering from disseminated intravascular coagulation and degenerative liver disease (e.g. liver cirrhosis). Also, vitronectin deposition is associated with atherosclerotic lesions.

PRINCIPLE OF THE METHOD

Diluted samples are added to microwells coated with a monoclonal antibody against Vitronectin. During an incubation period, Vitronectin present in the sample will bind to the antibody coated to the wells. Following a washing step, a horseradish peroxidase (HRP) conjugated rabbit anti-Vitronectin polyclonal antibody is added to the microwells and binds to the Vitronectin protein captured on the plate during a short incubation period. Following another washing step, the addition of a perborate-3,3'-5,5'-tetramethylbenzidine (TMB) substrate and its subsequent reaction with the HRP present generates a blue colored solution. The reaction is stopped by adding citrate stop solution, which turns the solution color yellow. Measuring the solution absorbance at 450 nm and extrapolating the value with those of a standard curve determines the level of Vitonectin in the diluted plasma sample.

REAGENTS

- MTP **Antibody Coated Microtiter plate,** MTP-96 (12x8) well
- WASH **Wash buffer**, 50 ml, 1 vial (concentrate)
- DILB **Dilution buffer**, 50 ml, 2 vials (ready-to-use)
- STD **Standard,** 1 ml human plasma, 1 vial (lyophilized)
- AB **Antibody conjugate**, HRP-conjugated anti-human Vitronectin, 140 µl, 1 vial (100x concentrate)
- TMB **Substrate**, 11 ml, 1 vial (ready-to-use)
- STOP **Stop solution**, 6 ml, 1 vial (ready-to-use)

PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human origin will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

Not for internal use in humans or animals. Do not use the kit components beyond the expiration date. Do not mix reagents from different kit lots. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth or ingest reagents. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Handle gently; avoid splashing, foam, or aerosol formation.

WASH DILB AB	Warning		H317, P280, P333+P313
STOP	Warning		H314, P303+P361+P353, P305+P351+P338, P310

Hazard Statements:
H317 May cause an allergic skin reaction.
H314 Causes severe skin burns and eye damage

Precautionary Statements:
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTRE or doctor/physician.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as instructed.

MTP **Antibody coated microwells:** Once removed from the foil pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture.

WASH **Wash buffer:** If crystals are visible, incubate the vial in a 37°C water bath a few minutes until the crystals are dissolved. Transfer the content to a 1 liter bottle and fill up the concentrate to 1 liter with filtered deionized/distilled water. Diluted Wash Buffer may be used for up to 4 weeks when stored at 2°-8°C.

DILB **Dilution buffer:** Supplied ready to use. Opened dilution buffer is stable for 3 month when stored at 2°-8°C.

STD **Standard:** Reconstitute the standard plasma with 1 ml purified, deionised or distilled water, swirl the contents gently and allow the vials to stand at room temperature for at least 15 minutes to ensure complete dissolution. The lyophilised standard plasma is stable until the date indicated on the vial label when stored at 2° - 8°C. Once reconstituted, the standard will remain stable for 3 month when stored at -20 °C.

AB **Antibody conjugate:** Supplied as a concentrate, dilute the Antibody conjugate 1:100 with Dilution buffer just prior to use. For using all 96 microwells at one time, dilute 100 µL of Antibody conjugate to 10 mL in Dilution Buffer. If not all 96 microwells are used, dilute 10 µL of Antibody conjugate to 1 mL in Dilution buffer for each 8-microwell strip that will be used. Working strength Antibody conjugate is stable for 4 hours at 2°-8°C. Discard any unused working strength Antibody conjugate. Opened antibody is stable for 3 month when stored in the dark at 2°-8°C.

TMB **Substrate, TMB:** Supplied ready to use. Opened substrate is stable for 3 month when stored in the dark at 2° - 8°C.

STOP **Stop solution:** Supplied ready to use. Opened stop solution is stable for 3 month when stored at 2° - 8°C.

SPECIMEN COLLECTION AND PREPARATION

Citrate, EDTA, or Heparin collected platelet poor plasma and serum may be used for this assay. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays; Approved Guidelines-Fourth Edition", NCCLS Document H21-A4, Vol. 23, No. 35, December 2003. Citrate plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 5,000 x g for 15 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at –20°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

0.22 µm filtered deionized H_2O
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
microwell plate reader for reading absorbance at 450 nm
microwell plate washer (optional), microwell plate shaker (optional)

Preparing the Vitronectin Standards

1. Reconstitute the standard plasma as instructed under REAGENT PREPARATION. Make serially dilutions of this Standard with Dilution Buffer as follows. Use Dilution Buffer as the 0 ng/ml standard:

Tube	Dilution	STD Lot 210718	Dilution buffer	Conc. [ng/ml]
Pre-dilution	1:10	10 µl Standard	90 µl	-
(1)	1:600	10 µl Pre-dilution	590 µl	100
(2)	1:1200	300 µl from (1)	300 µl	50
(3)	1:2400	300 µl from (2)	300 µl	25
(4)	1:4800	300 µl from (3)	300 µl	12,5
(5)	1:9600	300 µl from (4)	300 µl	6,25
(6)	1:19200	300 µl from (5)	300 µl	3.12
	-	-	300 µl	0

Preparing the Sample Dilutions

2. Dilute each plasma or serum sample 1:4000 with dilution buffer. It is recommended to make a 1:100 pre-dilution (5 µl sample + 495 µl dilution buffer) and then a 1:40 dilution (10 µl pre-diluted sample + 390 µl dilution buffer).

Running standard and samples in duplicate is recommended.

Assay Procedure

3. Open the foil pouch and remove the microwell strips/frame assembly. Remove the strips that will not be used, return them to the foil pouch and tightly reseal the pouch with the desiccant inside. Store the foil pouch at 2 - 8°C.

4. Pipette 100 µL of the diluted standard or samples into separate microwells, cover with the acetate sheet and incubate for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).

5. Empty the contents of the microwells and wash 4 times with Wash Buffer. Washing may be performed either using microwell plate washing equipment or manually (fill the wells with Wash Buffer with a pipette or squeeze bottle, wait three minutes, empty and remove droplets by tapping the plate 4-5 times face down against absorbing material).

6. Add 100 µL working strength Detection Antibody to each microwell, cover with the acetate sheet and incubate the wells for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).

7. Wash the wells by repeating Step 5.

8. Add 100 µL of Substrate to each microwell immediately after the wash step, cover the wells with the acetate sheet and incubate for approx. 5-10 minutes at room temperature (18°-25°C). A blue color will develop.

9. Stop the enzymatic reaction by adding 50 µL Stop solution to each microwell. Add the Stop solution with the same speed and order as you added the substrate. Tap the sides of the microwell frame to ensure even distribution of the solution. The solution color will turn yellow. Read the absorbance on a microwell plate reader at a wavelength of 450 nm within 10 minutes.

RESULTS

Construct a standard curve by plotting the mean absorbance value for each standard versus its corresponding concentration. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.

CALCULATIONS

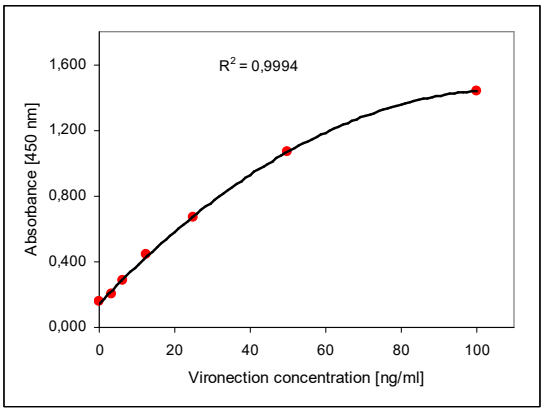
Determine the amount of Vitronectin in the diluted sample by interpolating directly from the standard curve. As the sample was diluted 1:4000 during its preparation, multiply the results by 4000 in order to obtain the concentration of Vitronectin in the neat plasma sample. The calculation is:

$$[\text{Vitronectin}]_{\text{Sample}} = [\text{Vitronectin}]_{\text{Diluted Test Sample}} \times 4000$$

LIMITATIONS OF THE PROCEDURE

Samples should not be collected with EDTA as the anticoagulant. Icteric, lipemic and hemolyzed samples may interfere with the assay.

Representative Standard Curve



LIMITATIONS OF THE PROCEDURE

Platelet contamination in plasma samples will interfere with the assay results. Plasma samples must be free of platelets in order to have a valid result. Exercise great care in minimizing disruption of the platelet pellet while recovering the platelet poor plasma. Samples should not be frozen and thawed more than two times.
Icteric, lipemic and hemolyzed samples may interfere with the assay.

PERFORMANCE CHARACTERISTICS

Specificity

The capture and detection antibodies are highly specific for human Vitronectin.

BIBLIOGRAPHY

- Vitronectin stabilizes thrombi and vessel occlusion but plays a dual role in platelet aggregation. Reheman A et al., J Thromb Haemost. 2005 May;3(5):875-883.
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- Role of vitronectin and its receptors in haemostasis and vascular remodeling. Preissner KT, Seiffert D. Thromb Res. 1998 Jan 1;89(1):1-21.