Catalog # HVNKT-TOT

Human Vitronectin Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This human vitronectin total assay is for the quantitative determination of total vitronectin in biological fluids.

BACKGROUND

Vitronectin is an abundant plasma glycoprotein that helps regulate coagulation, fibrinolysis, complement activation, and cell adhesion [1, 3, 8]. Vitronectin binds to glycosaminoglycans, collagen, plasminogen and urokinase receptors. It also may control the clearance of vascular thrombi by binding and stablilizing PAI-1. In binding PAI-1, it extends the lifetime of active PAI-1 [4, 5]. Vitronectin may also be involved in the regulation of bone metabolism [2].

ASSAY PRINCIPLE

Human vitronectin will bind to the capture antibody coated on the microtiter plate. Free and ligand bound enzyme will react with the antibody on the plate. After appropriate washing steps, polyclonal anti-human vitronectin primary antibody binds to the vitronectin. Excess antibody is washed away and bound polyclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. Following an additional washing step, TMB substrate is used for color development at 450nm. The amount of color development is directly proportional to the concentration of total vitronectin antigen in the sample.

REAGENTS PROVIDED

- Immunoassay plate:
  1-96 well immulon plate coated with anti human vitronectin capture antibody, blocked, and dried
- 10X Wash Buffer:
  1 bottle of 50ml wash; bring to 1X using DI water
- Human vitronectin antigen standard:
  1 vial of lyophilized standard
- Anti-human vitronectin primary antibody:
  1 vial of lyophilized polyclonal antibody
- Anti-rabbit secondary antibody:
  1 vial of concentrated horseradish peroxidase conjugated antibody
- TMB substrate solution:
  1 bottle of 10 ml solution

STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- Pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H_2SO_4
- DI water

- Magnetic stirrer and stir-bars
- Plastic containers
- ELISA plate cover
- TBS buffer
- 3% Blocking buffer
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

WARNINGS

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. DO NOT pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- All kit components must be kept refrigerated (4°C).
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris-HCl 0.15M NaCl, pH 7.4
- **Blocking buffer:** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Samples of human plasma, serum, urine, cell culture media, or tissue extracts may be applied directly to the plate.

The assay measures total vitronectin in the 0.05-100 ng/ml range. Samples giving vitronectin levels above 100 ng/ml should be diluted in a similar biological fluid devoid of vitronectin. Blocking buffer may also be used.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:

Reconstitute standard as directed on vial to give a 1,000 ng/ml standard solution.

Dilution table for preparation of human vitronectin standard:

Vitronectin concentration (ng/ml)	Dilutions
100	900µl (BSA) + 100µl (1,000 ng/ml from vial)
50	500µl (BSA) + 500µl (100 ng/ml)
20	600µl (BSA) + 400µl (50 ng/ml)
10	500µl (BSA) + 500µl (20 ng/ml)
5	500µl (BSA) + 500µl (10 ng/ml)
2	600µl (BSA) + 400µl (5 ng/ml)
1	500µl (BSA) + 500µl (2 ng/ml)
0.5	500µl (BSA) + 500µl (1 ng/ml)

NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:

Remove microtiter plate from bag and add 100µl of vitronectin standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess

wash by gently tapping plate on paper towel or kimwipe.

NOTE: If the unknown is thought to have high vitronectin levels, dilutions may be made in a similar biological fluid devoid of vitronectin, or in blocking buffer. A 1:10,000 dilution, generated by two serial dilutions of 1:100 each, is recommended for the measurement of vitronectin in normal human plasma.

Primary Antibody Addition:

Reconstitute primary antibody by adding 10ml blocking buffer to vial. Agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition:

Dilute 1µl conjugated secondary antibody in 10ml blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Quench the reaction with the addition of 50µl of 1N H_2SO_4 and read final absorbance values at 450nm.

NOTE: Time for substrate development is dependent on needs of researcher.

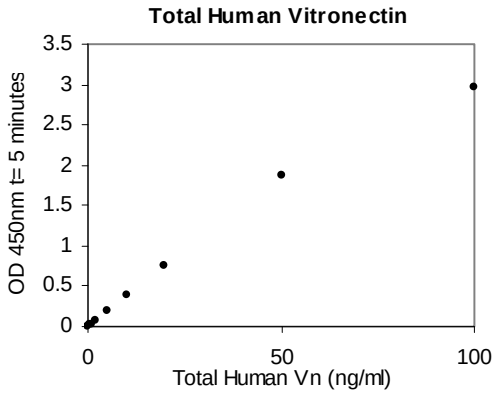
Measurement:

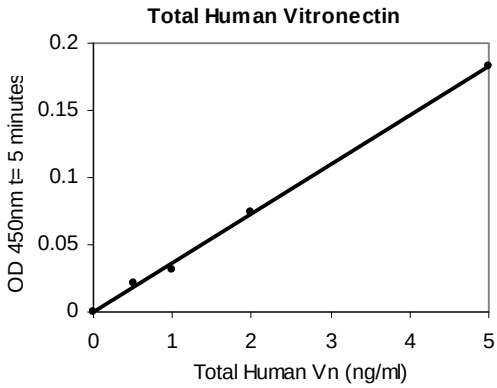
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A_{450}.

Assay Calibration:

Plot A_{450} against the amount of vitronectin in the standards. Fit a straight line through the points using a linear fit procedure. The vitronectin in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





EXPECTED VALUES

The concentration level vitronectin in human plasma or serum has been reported to be 200-400 µg/ml [7, 8].

Abnormalities in vitronectin levels have been reported in the following conditions:

- Coronary Artery Disease (CAD): It is suggested that vitronectin may be a marker of CAD and elevated levels may indicate a role in the genesis and/or progression of CAD [4].
- Platelet Aggregation: Vitronectin may have a physiological contribution to platelet aggregation on a blood clot. [6].

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

REFERENCES

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7. Colan MG, *et al*:: Plasma Vitronectin Polymorphism in Normal Subjects and Patients with Disseminated Intravascular Coagulation. Blood, Jul; **72(1)**:185-90, 1988.
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