Catalog # MVNKT-TOT

# Mouse Vitronectin Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

## For Research Use Only.

### INTENDED USE

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

### BACKGROUND

Vitronectin is an abundant plasma glycoprotein that helps regulate coagulation, fibrinolysis, complement activation, and cell adhesion [1,3,7]. 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

Mouse 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, biotinylated polyclonal anti-mouse vitronectin primary antibody binds to the vitronectin. Excess antibody is washed away and bound polyclonal antibody is then reacted with avidin 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 in the sample.

### REAGENTS PROVIDED

- Immunoassay plate:
  1-96 well immulon plate (8X12 strips removable wells) coated with anti mouse vitronectin capture antibody, blocked, and dried
- 10X Wash Buffer:
  1 bottle of 50ml wash; bring to 1X using DI water
- Mouse vitronectin standard:
  1 vial lyophilized standard
- Anti-mouse vitronectin primary antibody:
  1 vial of lyophilized biotinylated polyclonal anti-mouse vitronectin antibody
- Avidin peroxidase secondary reagent:
  1 vial of concentrated HRP labeled avidin
- 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 mouse urine, cell culture media, or tissue extracts may be applied directly to the plate. Samples of mouse plasma and serum should be diluted 1:100 to 1:1,000 in blocking buffer before applying them to the plate.
The assay measures total vitronectin in the 5ng/ml-1000ng/ml range.

## 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 1,000ng/ml standard solution.

Dilution table for preparation of mouse vitronectin standard:

| Vitronectin concentration (ng/ml) | Dilutions |
|---|---|
| 1000 | Straight from vial |
| 500 | 500µl (BSA) + 500µl (1000ng/ml) |
| 200 | 600µl (BSA) + 400µl (500ng/ml) |
| 100 | 500µl (BSA) + 500µl (200ng/ml) |
| 50 | 500µl (BSA) + 500µl (100ng/ml) |
| 20 | 600µl (BSA) + 400µl (50ng/ml) |
| 10 | 500µl (BSA) + 500µl (20ng/ml) |
| 5 | 500µl (BSA) + 500µl (10ng/ml) |
| 0 | 500µl (BSA) Zero point to determine background |

**NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD 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 plasma devoid of vitronectin, or in blocking buffer.

**Primary Antibody Addition:**
Reconstitute primary antibody as directed on vial and 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.

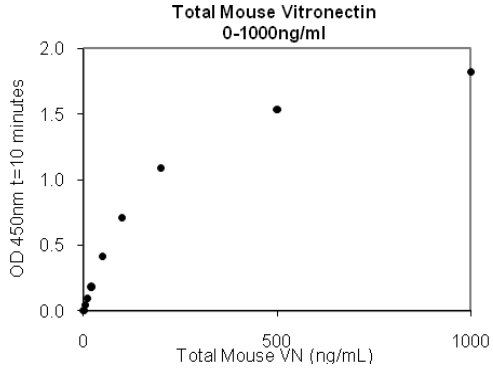
**Secondary Reagent Addition:**
Dilute 2.5µl into 10ml blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

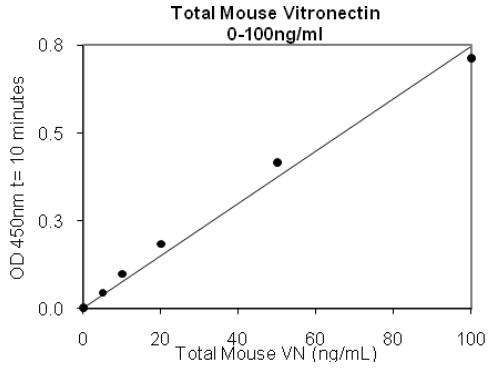
**Substrate Incubation:**
Add 100µl TMB substrate to all wells and shake plate for 10-15 minutes. Quench the reaction with the addition of 50µl of 1N $H_2SO_4$ and read final absorbance values at 450nm.
**NOTE:** Time for substrate development is dependent on needs of researcher.

**Measurement:**
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance ($A_{450}$).

**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 vitronectin concentration in mouse serum has been estimated at 300 µg/ml by semi-quantitative immunoblotting [8].

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

- 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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3. Dufourcq P, *et al.*: Vitronectin Expression and Interaction With Receptors in Smooth Muscle Cells From Human Atheromatous Plaque. Thromb. Vas Biol, **18**:168-176, 1998.
4. Ekmemci M, *et al.*: Plasma Vitronectin Levels in Patients with Coronary atherosclerosis are increased and Correlate with Extent of Disease. J Thromb Thrombolysis, **14(3)**:221-5, 2002.
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7. Bittorf SV, *et al.*: Alteration of Vitronectin. Characterization of changes induced by treatment with urea. J Biol Chem, Nov 25; **268(33)**:24828-24846, 1993.
8. Seiffert D, *et al.*: Detection of Vitronectin mRNA in Tissues and Cells of the Mouse. PNAS, Nov;**88**:9402-9406, 1991.