LSBio™ Mouse VTN / Vitronectin ELISA Kit

Catalog No. LS-F10454

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio
LifeSpan BioSciences, Inc.

For research use only. Not approved for use in humans or for clinical diagnosis.

Mouse Vitronectin Total Antigen ELISA Kit

Catalog # LS-F10454 Strip well format. Reagents for up to 96 tests.

www.lsbio.com

Rev: December 2014

INTENDED USE

This mouse vitronectin total antigen assay is for the quantitative determination of total vitronectin antigen in biological fluids. **For research use only.**

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 stabilizing 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 affinity purified 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 anti-mouse vitronectin primary antibody binds to the captured protein. Excess antibody is washed away and bound polyclonal antibody is reacted with peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of mouse vitronectin. The amount of color development is directly proportional to the concentration of total vitronectin in the sample.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse vitronectin antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Mouse Vitronectin standard:** 1 vial lyophilized standard
- **Anti-mouse Vitronectin primary antibody:** 1 vial lyophilized biotinylated polyclonal antibody
- **Horseradish peroxidase-conjugated Streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

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

Preparation of Standard

Reconstitute standard by adding 1.5ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000ng/ml standard solution.

Dilution table for preparation of mouse vitronectin standard:

Vitronectin concentration (ng/ml)	Dilutions
100	900µl (BB) + 100µl from vial
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0	500µl (BB) 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 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: The assay measures total mouse vitronectin antigen in the 1-100ng/ml range. Samples with mouse vitronectin levels above 100ng/ml should be diluted in blocking buffer before use. If the unknown is thought to have high vitronectin levels, dilutions may be made in blocking buffer. A 1:10,000-1:40,000 dilution for normal mouse plasma or serum is suggested for best results. Samples of mouse urine, cell culture media, or tissue extracts may be applied directly to the plate.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the 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.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100µl of the 1:50,000 dilution 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 1-5 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

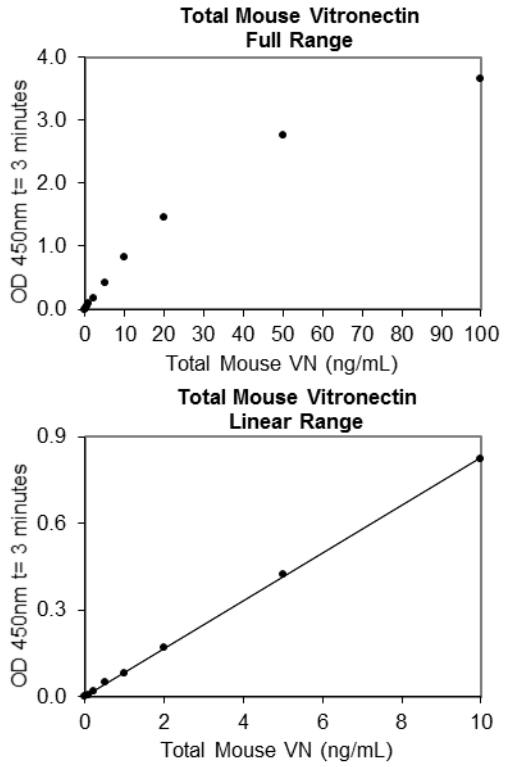
Measurement

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}).

Calculation of Results

Plot A_{450} against the amount of vitronectin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of vitronectin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



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].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.071-0.083) and calculating the corresponding concentration. The MDD was 0.026ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

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 of or contact with the above product.

REFERENCES

1.Podor TJ, *et al.:* J Biol Chem. 2001, **277**(9):7520-8.
2.Seiffert D: J. Histochem Cytochem. 1996, **44**(3):275-80.
3.Dufourcq P, *et al*.:Thromb. Vas Biol. 1998, **18**:168-176.
4.Ekmemci M, *et al*.:J Thromb Thrombolysis. 2002, **14(3)**:221-5.
5.Zhou A,*et al*.: Nat Struct Biol. 2003, Jul **10(7)**:541-4.
6.Wu YP,*et al*.:Blood. 2004, **104(4)**:1034-41.
7.Bittorf SV, *et al*.: J Biol Chem. 1993, **268(33)**:24828-24846.
8.Seiffert D, *et al.*: PNAS. 1991, **88**:9402-9406.

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	
B	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	
C												
D												
E F												
G												
H												

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion.

Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

Returns, Refunds, Cancelations: Any problems with LifeSpan products must be reported to LifeSpan within 10 days of product receipt. The customer must obtain written authorization from LifeSpan before returning items. To request that goods be returned, please contact LifeSpan Technical Support. If an error by LifeSpan BioSciences results in shipment of an incorrect order, LifeSpan will, at its option, either ship a replacement order at no charge, or credit the customer's account for the original product shipped in error. Returns and cancelations may be subject to a 30% restocking fee.

Conditions & Warranty: All LifeSpan products are intended for Research Use Only and are not for use in human therapeutic or diagnostic applications. The information supplied with each product is believed to be accurate, but no warranty or guarantee is offered for the products, because the ultimate conditions of use are beyond LifeSpan's control. The information supplied with each product is not to be construed as a recommendation to use this product in violation of any patent, and LifeSpan will not be held responsible for any infringement or other violation that may occur with the use of its products. Under no event will LifeSpan be responsible for any loss of profit or indirect consequential damage, including, but not limited to, personal injuries resulting from use of these products. LifeSpan's liability to any user of Products for damages that do not result from any fault of the user, will be limited to replacement of the Product(s) only, and in no event shall LifeSpan's liability exceed the actual price received by LifeSpan for the Product(s) at issue. LifeSpan shall not be liable for any indirect, special, incidental or consequential damages. LIFESPAN FURTHER DISCLAIMS ANY AND ALL EXPRESS AND IMPLIED OR STATUTORY WARRANTIES WITH RESPECT TO THE PRODUCTS, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE. LifeSpan disclaims any and all responsibility for any injury or damage which may be caused by the fault of the user.

For research use only. Not approved for use in humans or for clinical diagnosis.

LSBio
LifeSpan BioSciences, Inc.

2401 Fourth Avenue Suite 900 Seattle, WA 98121
Tel: 206.374.1102
Fax: 206.577.4565

Technical.Support@LSBio.com