LSBio™ Human PAI-1 + tPA Complex ELISA Kit

Catalog No. LS-F10422

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.

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www.lsbio.com

Human PAI-1/tPA Complex Antigen ELISA Kit

Catalog # LS-F10422 Strip well format. Reagents for up to 96 tests.

Rev: December, 2014

INTENDED USE

Human PAI-1/tPA complex assay is intended for the quantitative determination of the covalent complex of tPA and its inhibitor PAI-1 in human plasma, serum, urine, cell culture media, or tissue extracts. **For research use only.**

BACKGROUND

Tissue-type plasminogen activator (tPA) is a serine protease that activates plasminogen to plasmin in the blood fibrinolytic system. Plasminogen activator inhibitor type 1 (PAI-1) is involved in the regulation of the blood fibrinolytic system and forms a 1:1 covalent complex with uPA and tPA.

ASSAY PRINCIPLE

Human PAI-1 in samples will bind to the monoclonal capture antibody coated on the microtiter plate. Free, latent, and complexed PAI-1 will react with the capture antibody. After appropriate washing steps, polyclonal anti-human tPA primary antibody binds to PAI-1/tPA complex. Excess antibody is washed away and bound polyclonal antibody is then reacted with the peroxidase conjugated secondary antibody.

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 PAI-1/tPA complex. Color development is proportional to the concentration of PAI-1/tPA complex in the samples.

Free tPA and PAI-1 will not be detected by this assay.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human PAI-1 antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human PAI-1/tPA complex 0ng standard:** 2 vials lyophilized PAI-1 depleted plasma
- **Human PAI-1/tPA complex 200ng standard:** 1 vial lyophilized PAI-1 depleted plasma with 200ng PAI-1/tPA complex
- **Anti-human tPA primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-rabbit horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **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 0ng standards by adding 1ml de-ionized water directly to the vial. Agitate gently to completely dissolve contents. This will result in a 0ng/ml standard solution. Reconstitute 160ng standard by adding 1ml of de-ionized water directly to the vial. Agitate gently to completely dissolve contents. This will result in a 160ng/ml standard solution.

Dilution table for preparation of human PAI-1/tPA complex standard:

PAI-1/tPA complex concentration (ng/ml)	µl of 200 ng/ml PAI-1/tPA complex standard	µl of 0 ng/ml PAI-1/tPA complex standard	Total volume (µl)
100	150	90	240
50	75	165	240
20	30	210	240
10	15	225	240
5	7.5	232.5	240
2	3	237	240
1	2.4 of 100	237.6	240
0.5	2.4 of 50	237.6	240
0	0	220	220

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 PAI-1/tPA complex 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 PAI-1/tPA antigen in the 0.5-100 ng/ml range. If the unknown is thought to have high PAI-1/tPA complex levels, dilutions may be made in plasma devoid of PAI-1 or in additional zero unit standard.

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.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2µl of conjugated secondary antibody in 10ml of 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 2-5 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.

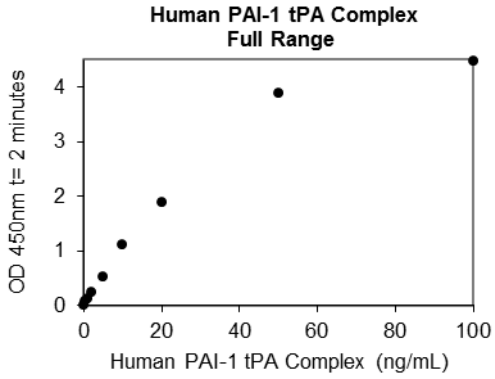
Measurement

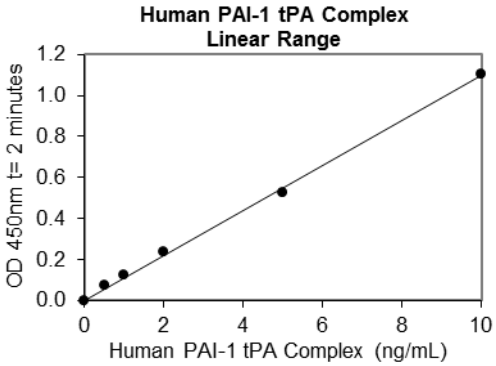
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of PAI-1/tPA complex 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 PAI-1/tPA complex in the unknowns can be determined from this curve. If samples have been dilute d, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

Concentration of PAI-1/tPA complex in normal human plasma was found to be 2.8±1.6 ng/ml and increases during pregnancy, artherosclerosis, and sepsis [1]. Complex levels are similar in males (5.77±3.07 ng/ml, n=189) and females (5.23±2.85 ng/ml, n=189) and are associated with myocardial infarction reoccurrence [2]. High levels of PAI-1/tPA complex in breast cancer cytosols are associated with poor survival [3]. PAI-1/tPA complex levels may also be useful as a prognostic indicator for renal/bladder cancer [4], multiple organ failure [5], and stroke [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 OD450: 0.06-0.074) and calculating the corresponding concentration. The MDD was 0.03ng/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: This assay recognizes natural and recombinant total human PAI-1-tPA Complex. Pooled normal plasma from cyno monkey and horse was assayed and minor cross-reactivity was observed. Pooled normal plasma from mouse, rat, pig, dog and sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from rabbit resulted in significant color development.

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. Fareed J, *et al.*: Clin Chem. 1998, 44:1845-1853.
2. Wiman B, *et al.*: Arterioscler Thromb Vasc Biol. 2000, 20:2019-2023.
3. de Witte JH, *et al.*: Br J Cancer. 1999, 80:286-294.
4. Span PN, *et al.*: BJU Int. 2008, 102:177-182.
5. Hoshino M, *et al.*: Crit Care. 2001, 5:88-99.
6. Johansson L, *et al.*: Stroke. 2000, 31:26-32.

Example of ELISA Plate Layout
96 Well Plate: 18 Standard wells, 78 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	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	0.5 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.

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