LSBio™ Rat PLAT / TPA Enzyme Capture ELISA Kit

Catalog No. LS-F10477

User Manual

Please Read the Manual Carefully Before Starting your Experiment

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

LSBio
LifeSpan BioSciences, Inc.
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Rat tPA Activity ELISA Kit

Catalog # LS-F10477 Strip well format.
Reagents for up to 96 tests.

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INTENDED USE

This rat tPA activity assay is intended for the quantitative determination of active tissue plasminogen activator (tPA) in rat plasma and other biological fluids. **For research use only.**

BACKGROUND

tPA is a serine protease that converts plasminogen to the active serine protease plasmin in the blood fibrinolytic system [1,2]. It also plays an important role in the removal of incipient thrombi [3]. tPA is widely used for the thrombolytic treatment of acute myocardial infarction [3].

ASSAY PRINCIPLE

Functionally active rat tPA will form a covalent complex with the biotinylated human PAI-1 which is bound to the avidin on the plate. Only free active tPA will react with the PAI-1 bound to the plate. After appropriate washing steps, polyclonal anti-tPA primary antibody binds to the captured tPA. Excess antibody is washed away and bound primary 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 active tPA in the sample.

REAGENTS PROVIDED

- **96-well avidin coated microtiter strip plate** (removable wells 8x12) containing avidin, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **10X TBS buffer, pH 7.4:** 1 bottle of 5mL
- **Biotinylated Human PAI-1:** 1 vial lyophilized protein
- **Rat tPA activity standard:** 1 vial lyophilized standard
- **Anti-tPA primary antibody:** 1 vial lyophilized monoclonal antibody
- **Anti-mouse 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 or citrate as an anticoagulant. Heparinated plasma is not recommended. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. It is important to ensure a platelet free preparation as platelets can release PAI-1, which in turn could potentially form a complex with tPA. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles. Serum and cell culture media at neutral pH may also be used.

ASSAY PROCEDURE

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

Biotinylated Human PAI-1 Addition

Add 10ml blocking buffer directly to the biotinylated human PAI-1 vial and agitate gently to completely dissolve contents. Remove microtiter plate from bag 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.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Dilution table for preparation of rat tPA standard:

tPA concentration (ng/ml)	Dilutions
50	950µl (BB) + 50µl (from vial)
25	500µl (BB) + 500µl (50ng/ml)
10	600µl (BB) + 400µl (25ng/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)
0.25	500µl (BB) + 500µl (0.5ng/ml)
0.1	600µl (BB) + 400µl (0.25ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/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

If using acidified citrate samples with a pH lower than 6.0, add 30µl of 10X TBS buffer in each well and construct the standard curve in the same format. If using samples at a neutral pH, this step can be omitted. Add 100µl tPA 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 active tPA in the 0.05-50 ng/ml range. If the unknown is thought to have high tPA levels, dilutions may be made in blocking buffer. Plasma and serum samples should be applied directly to the plate without dilution.

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 1µl 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 2-10 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.

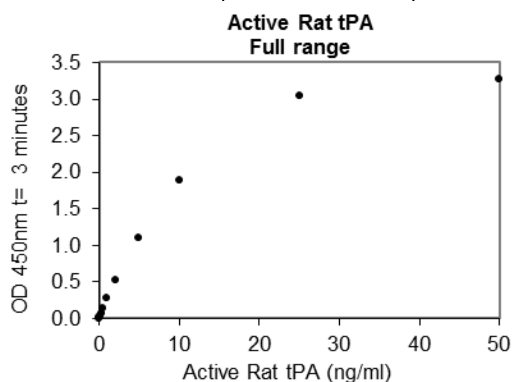
Measurement

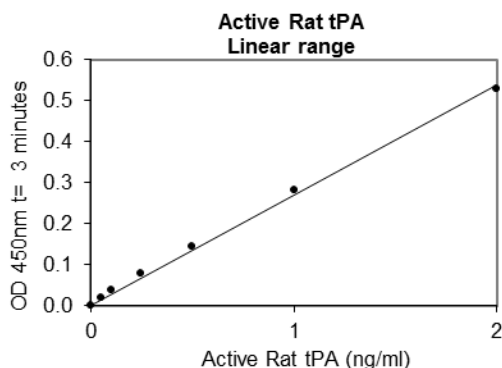
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of tPA 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 tPA 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

NOTE: No specific data has been reported for rat tPA concentrations. Please refer to references for mouse tPA. The concentration level of tPA antigen in mouse plasma has been reported to be 2.5±1.0 ng/mL [4].

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

- Ischemic Diseases: tPA may affect the course of ischemic diseases [5].
- Pathological Infarction: tPA may prevent or limit pathological infarction and improve neurological functions [6]. Usage of tPA at the onset of ischemic stroke improves clinical outcome [7].
- Blood-Brain Barrier: tPA is necessary and sufficient to directly increase the vascular permeability in the early stages of BBB opening [8].
- Venous Thrombosis: Locally applied tPA reduces thrombus formation after vascular injury [9].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty-two zero standard replicates (range OD_{450}: 0.059-0.071) and calculating the corresponding concentration. The MDD was 0.043ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.80	4.25	13.2
Standard Deviation	0.034	0.225	0.553
CV (%)	4.28	5.29	4.20

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	1.37	4.80	13.3
Standard Deviation	0.06	0.34	0.75
CV (%)	4.71	7.13	5.65

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.20	0.45	1.43	6.19
Average % Recovery	102	106	97	90
Range	98-105%	94-118%	93-106%	86-100%

Linearity: To assess the linearity of the assay, pooled citrated rat plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay. Plasma samples did not demonstrate linearity and should be analyzed without dilution.

Specificity: This assay recognizes natural and recombinant active rat tPA. Pooled normal plasma from mouse was assayed and significant cross-reactivity was observed. Pooled normal plasma from pig was assayed and minor cross-reactivity was observed. Pooled normal plasma from human, horse, dog, rabbit and sheep was assayed and no significant cross-reactivity was observed.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (ng/mL)
Citrate Plasma	Undiluted	7.9

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

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5. Nassar T, *et al.*: Blood. 2003, 103(3):897-902.
6. Sakurama T, *et al.*: Stroke. 1994, 25:451-456.
7. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue Plasminogen Activator for Acute Ischemic Stroke. N Engl J Med. 1995, 333(24):1581-1588.
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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	0.05 ng/ml	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	
B	0	0.05 ng/ml	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 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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