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Mouse tPA Activity ELISA Kit

Catalog # MTPAKT

Strip well format. Reagents for up to 96 tests.

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INTENDED USE

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

BACKGROUND

Tissue plasminogen activator (tPA) is a serine protease that converts plasminogen to plasmin in the blood fibrinolytic system [1,2,3,9]. It also plays an important role in the nervous system, including the processes of neuronal migration, neurite outgrowth, and neuronal plasticity [1,2,4,7,10]. tPA has been suggested to have a role in several neuropathological conditions such as cerebral ischemia, seizures, and demyelinating diseases [1,3,5].

ASSAY PRINCIPLE

Functionally active mouse tPA will bind to the biotinylated human PAI-1 coated on the microtiter plate. Only free active enzyme will react with the PAI-1 on the plate. A standard calibration curve is prepared using dilutions of tPA along with the samples to be measured. After appropriate washing steps, monoclonal anti-mouse tPA primary antibody binds to the captured enzyme. Excess antibody is washed away and bound monoclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. Following an additional washing step, TMB 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
- **Biotinylated Human PAI-1:** 1 vial lyophilized protein
- **10X TBS Buffer:** 1 vial of 5ml
- **Mouse tPA activity standard:** 1 vial lyophilized standard
- **Anti-mouse 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 standard 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 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate. Immediately after collection of blood, samples must be centrifuged at 3000Xg for 15 minutes. 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. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to analysis. The tPA activity samples are stable for up to 24 hours on ice, up to one month frozen at –20°C, or up to 5 months at -70°C. tPA activity samples can be thawed three times without loss of tPA activity. Samples must be at a neutral pH to be used in the assay. If samples were collected in citrate, the pH should be adjusted with the 10X TBS provided in the kit as described below.

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 mouse tPA standard:

tPA concentration (ng/ml)	Dilutions
50	950µl (BB) + 50µl (1000ng/ml)
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 antigen 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 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 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.

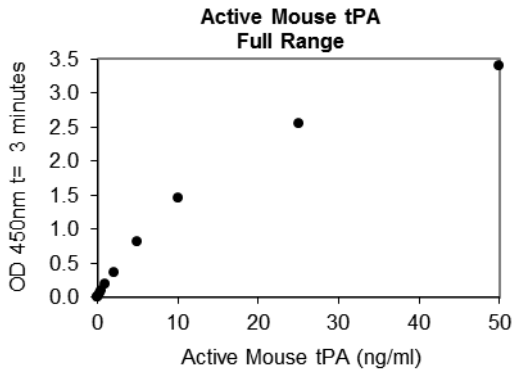
Measurement

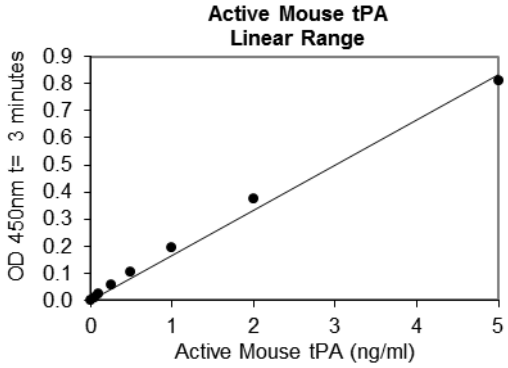
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of 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

The concentration level of endogenous tPA antigen in murine plasma has been reported to be 2.5+/-1.0 ng/ml [15].

In house testing of pooled normal mouse plasma in citrate indicates tPA levels vary by mouse strain:

Strain	Active tPA	Total tPA
NSA/CF-1	9.9 ng/ml	9.4 ng/ml
C57BL6	1.4 ng/ml	2.4 ng/ml
CD-1	0.4 ng/ml	0.4 ng/ml

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

- Venous Thrombosis: Endogenous tPA plays a key role in restoring cerebral blood flow and limiting infarct size after thrombosis [6].
- Spinal Cord Contusion: Suppression of tPA production may help decrease secondary injury after spinal cord injury [1].
- Ischemic Diseases: tPA may attenuate neuronal injury after mild focal cerebal ischemia [5]. tPA may be involved in the regulation of blood vessel tone, which may affect the course of ischemic diseases [3].
- Bone Formation: A decreased in tPA may result in an increase of bone formation [14].
- Diabetic Retinopathy: Increased tPA levels have been associated with proliferative diabetic retinopathy [8].
- Adipose Tissue Development: A decrease in tPA may increase the development of adipose tissue in diet-induced obesity [11].
- Stress-induced Anxiety: tPA is critical for the development of anxiety-like behavior after stress [12].

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}: 0.057-0.067) and calculating the corresponding concentration. The MDD was 0.014 ng/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.

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

Sample Type	Dilution	Mean (ng/mL)
Citrate Plasma	Undiluted	3.3
EDTA Plasma	Undiluted	9.8

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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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												