Rat tPA (active) ELISA Kit

Enzyme Immunoassay kit for the quantification of Rat tPA (active) in Rat plasma.

Catalog number: ARG81068

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INTRODUCTION

This gene encodes tissue-type plasminogen activator, a secreted serine protease which converts the proenzyme plasminogen to plasmin, a fibrinolytic enzyme. Tissue-type plasminogen activator is synthesized as a single chain which is cleaved by plasmin to a two chain disulfide linked protein. This enzyme plays a role in cell migration and tissue remodeling. Increased enzymatic activity causes hyperfibrinolysis, which manifests as excessive bleeding; decreased activity leads to hypofibrinolysis which can result in thrombosis or embolism. Alternative splicing of this gene results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Jul 2008]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique for the detection of Rat tPA (active) in plasma. Biotinylated Human PAI-1, which will bind to functionally active tPA, but not complexed tPA, will bind on avidin - coated microplate. Standards or samples are pipetted into the wells and any functionally active tPA present is bound on the plate. After washing away any unbound substances, a primary antibody binds to Rat tPA is added to each well and incubate. Following a washing to remove unbound substances, a secondary antibody conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of functionally active tPA bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The

concentration of functionally active tPA in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
avidin coated microplate	12 X 8 strips	4°C
Biotinylated Human PAI-1	1 vial (lyophilized)	4°C
10X TBS buffer (pH7.4)	5 ml	4°C
Standard	1 vial (lyophilized)	4°C, store at -80 °C after reconstitution
Anti-tPA Primary Antibody	1 vial (lyophilized)	4°C, store at -80 °C after reconstitution
HRP-conjugated secondary antibody	1 vial	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	10 ml (ready-to-use)	4°C (Protect from light)

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 1N H_2SO_4 or 1N HCl
- Blocking buffer (BB): 3% BSA (w/v) in TBS buffer
- TBS buffer: 0.1M Tris, 0.15M NaCl, pH 7.4
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)
- Orbital shaker

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reconstituted standards and primary antibody can be stored at -80°C for later use and avoid repeat freeze-thaw for more than once.
- Store the other unused kit components at 4°C at all times.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma: Collect plasma using EDTA or citrate as an anticoagulant (blood: anticoagulant = 9:1). Heparinized 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. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to analysis. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Samples must be at a neutral pH to be used in the assay. Samples of Rat plasma in citrate or EDTA may be assayed with this kit. Plasma in heparin is not recommended. Serum and cell culture media at neutral pH may also be used.

REAGENT PREPARATION

- **Standard**: Reconstitute standards by adding 1 ml of Blocking buffer (BB) directly to the vials and agitate gently to completely dissolve contents. This will result in a 1000 ng/ml standard solution. The Blocking buffer (BB) serves as zero standard (0 ng/ml)

  Dilution table for preparation of Rat tPA standard was as below:

tPA Concentration (ng/ml)	Dilution
50	950 µl (BB) + 50 µl (1000 ng/ml)
25	500 µl (BB) + 500 µl (50 ng/ml)
10	600 µl (BB) + 400 µl (25 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2	600 µl (BB) + 400 µl (5 ng/ml)
1	500 µl (BB) + 500 µl (2 ng/ml)
0.5	500 µl (BB) + 500 µl (1 ng/ml)
0.25	500 µl (BB) + 500 µl (0.5 ng/ml)
0.1	600 µl (BB) + 400 µl (0.25 ng/ml)
0.05	500 µl (BB) + 500 µl (0.1 ng/ml)
0	500 µl (BB) Zero point to determine background

  Note: Blocking buffer (BB): 3% BSA (w/v) in TBS

  Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.

- **1X Wash buffer**: Dilute 50 ml of 10X wash buffer concentrate with 450 ml of deionized water.
- **Biotinylated Human PAI-1:** Reconstitute Biotinylated Human PAI-1 by adding 10 ml of Blocking buffer (BB) directly to the vials and agitate gently to completely dissolve contents.
- **Primary Antibody:** Reconstitute primary antibody by adding 10 ml of

Blocking buffer (BB) directly to the vials and agitate gently to completely dissolve contents.

- **HRP-Conjugated secondary antibody:** Briefly centrifuge vial before opening. Dilute 1 µl of HRP conjugated secondary antibody into 10 ml blocking buffer to generate a 1:10,000 dilution.
- **Sample:** If the measuring absorbance of samples is higher than the highest standard, dilute the samples with blocking buffer before assay and assay again. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of Biotinylated Human PAI-1 into all wells. Shake plate at 300rpm for 30 minutes at RT.
3. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining buffer by aspirating, decanting or blotting against clean paper towels
4. If samples are at neutral pH:

   Add 100 µl of standards, samples and zero controls into appropriate wells. Shake plate at 300rpm for 30 minutes at RT.

   If the pH of samples is below pH 6.0 (if using acidified citrate as

anticoagulant):

Add 30 µl of 10X TBS buffer to all wells then add 70 µl of standards, samples and zero controls into appropriate wells. Shake plate at 300rpm for 30 minutes at RT.

5. Wash as according to step 3.
6. Add 100 µl of working primary antibody into each well. Shake plate at 300 rpm for 30 minutes at RT.
7. Wash as according to step 3.
8. Add 100 µl of working HRP-conjugated Secondary antibody (1:10,000 diluted) into each well. Shake plate at 300 rpm for 30 minutes at RT.
9. Wash as according to step 3.
10. Add 100 µl of TMB substrate to each well. Incubate for 2-10 minutes at RT in dark. Substrate will change from colorless to different strengths of blue.
11. Add 50 µl of 1N H_2SO_4 or HCl to each well. The color of the solution should change from blue to yellow. Mix thoroughly by gently shaking the plate.
12. Read the OD with a microplate reader at 450 nm immediately.

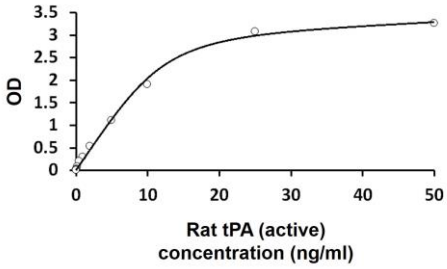
CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. 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.
6. Abnormalities in tPA levels have been reported in the following conditions:
   - Ischemic Diseases: tPA may affect the course of ischemic diseases.
   - Pathological Infarction: tPA may prevent or limit pathological infarction and improve neurological functions. Usage of tPA at the onset of ischemic stroke improves clinical outcome.
   - Blood-Brain Barrier: tPA is necessary and sufficient to directly increase the vascular permeability in the early stages of BBB opening.
   - Venous Thrombosis: Locally applied tPA reduces thrombus formation after vascular injury.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

Standard range: 0.05 – 50 ng/ml

Minimum Detectable Concentration: 0.043 ng/ml

Specificity

This assay recognizes natural and recombinant active rat tPA.

This assay resulted in significant color development with plasma from mouse.

Minor cross-reactivity was observed with plasma from pig.

No significant cross-reactivity or interference with the sample at below was observed: Pooled normal plasma from human, horse, dog, rabbit and sheep.

Precision:

Inter-assay Precision: 4.71-7.13 %

Intra-assay Precision: 4.20-5.29 %

Recovery:

86-118 %