

## Rabbit Active tPA ELISA Kit

**Catalog No:** CSI20542A    **Lot No:** tbd    **Size:** 1 Plate (1x 96 tests)    **Expiration Date:** 1 year from receipt

<b>Specificity:</b>	Functionally active rabbit tissue plasminogen activator
<b>Sensitivity:</b>	0.181 ng/mL    minimum detectable dose (MDD)
<b>Range:</b>	0.5-100 ng/mL
<b>Sample Type:</b>	Plasma, serum, cell culture media.
<b>Cross-Reactivity</b>	No significant cross reactivity with human, rat or porcine tPA

### Background:

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

### Assay Principle:

Functionally active rabbit tPA will form a covalent complex with biotinylated human PAI-1 bound to the avidin-coated microtiter plate. Only free active tPA will react with the PAI-1 bound to the plate. A standard calibration curve is prepared using dilutions of known tPA along with the samples to be measured. After appropriate washing steps, polyclonal anti-rabbit tPA primary antibody binds to the captured enzyme. Excess antibody is washed away and bound polyclonal 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:

Description	Quantity
<b>CSI20542A – P.</b> 96-well microtiter strip plate coated with avidin on well surface, blocked and dried	1 plate: 96 wells (12 strips x 8 wells)
<b>CSI20542A - A.</b> Wash buffer concentrate (10x)	1 bottle, 50 mL
<b>CSI20542A - B.</b> TBS buffer concentrate (10x)	1 bottle, 5 mL
<b>CSI20542A - C.</b> Biotinylated human PAI-1, xxxx ng/vial, lyophilized	1 vial
<b>CSI20542A - D.</b> Rabbit tPA activity standard, lyophilized.	1 vial
<b>CSI20542A – E.</b> Anti-tPA primary antibody, lyophilized	1 vial
<b>CSI20542A - F.</b> Anti-sheep horseradish peroxidase secondary antibody, concentrated	1 vial
<b>CSI20542A - G.</b> TMB substrate solution	1 bottle, 10 ml



## Rabbit Active tPA ELISA Kit

### Storage and Stability:

- Kit components should be stored at 2-8°C upon arrival.
- Store unopened plate and any unused microtiter strips in the pouch with desiccant.
- Reconstituted standards and primary may be stored at -80 °C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once.
- All other unused kit components must be stored at 2-8°C.
- Kit should be used no later than the expiration date.

### Reagents and Equipment 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<sub>2</sub>SO<sub>4</sub> 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 Lot. This kit is designed to work properly as provided within the Lot number.
- **DO NOT** pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- Always pour peroxidase substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink or eat in areas where specimens or reagents are being handled.

### Preparation of Reagents:

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



## Rabbit Active tPA ELISA Kit

### Sample Collection:

Collect plasma using EDTA or citrate as an anticoagulant. 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. Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . 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 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

#### Preparation of Standard:

Reconstitute standard as by adding **8 mL of Blocking Buffer (BB)** directly to the vial and agitate gently to completely dissolve contents. This will result in a **100 ng/mL** standard solution.

Prepare dilution series as indicated in the dilution table (at right) for preparation of rabbit tPA activity standard.

**NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.**

**NOTE:** The assay measures active tPA in the 0.5-100 ng/ml range. If the unknown is thought to have high tPA levels, dilutions may be made in blocking buffer.

#### Standard and Unknown Addition:

If using acidified citrate samples with a pH lower than 6.0, add 30 $\mu\text{l}$  of 10X TBS buffer to each well and construct the standard curve in the same format. If using samples at a neutral pH, this step can be omitted. Add 100 $\mu\text{l}$  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 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Rabbit tPA concentration (ng/ml)	Dilutions
100	100 $\mu\text{l}$ straight from vial
50	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (100ng/ml)
20	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (50ng/ml)
10	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (20ng/ml)
5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (10ng/ml)
2	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (5ng/ml)
1	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (2ng/ml)
0.5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (1ng/ml)
0	500 $\mu\text{l}$ (BB) Zero point to determine background



## Rabbit Active tPA ELISA Kit

### Primary Antibody Addition:

Reconstitute primary antibody by adding **10 mL of Blocking Buffer (BB)** directly to the vial and agitate gently to completely dissolve contents. Add 100  $\mu$ L to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300  $\mu$ L wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

### Secondary Antibody Addition:

Briefly centrifuge vial before opening. Dilute **2  $\mu$ L of conjugated secondary antibody into 10 mL Blocking Buffer (BB)** and add 100  $\mu$ L to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300  $\mu$ L wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

### Substrate Incubation:

Add 100  $\mu$ L 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 50  $\mu$ L of 1N H<sub>2</sub>SO<sub>4</sub> 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:

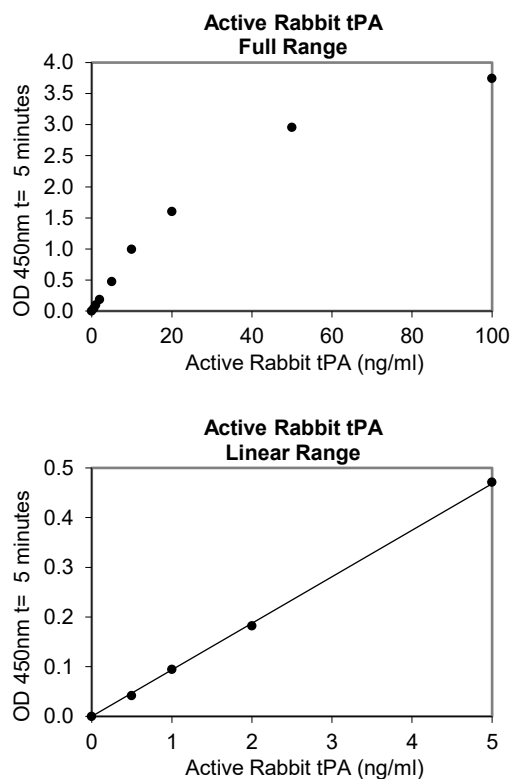
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. Subtract zero point from all standards and unknowns to determine corrected absorbance ( $A_{450}$ ).

### Calculation of Results:

Plot  $A_{450}$  against the amount of tPA in the standards. Fit a straight line through the 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):



## Rabbit Active tPA ELISA Kit

### 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<sub>450</sub>: 0.138-0.163) and calculating the corresponding concentration. The MDD was 0.181 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:** This assay recognizes rabbit tPA. Pooled normal plasma from human, rat, and pig was assayed and no significant cross-reactivity was observed. Pooled normal plasma from sheep resulted in significant color development.

### Example of ELISA Plate Layout

96 Well Plate: 18 Standard wells, 78 Sample \

	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												

**NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.**

