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Mouse uPA Total Antigen ELISA Kit

(Strip well format. Reagents for up to 96 tests.)

Catalog No.	**CSI19904A**	**Quantity:**	**1 x 96 tests**
	CSI19904B		**5 x 96 tests**

Intended Use: Mouse uPA total antigen assay is intended for the quantitative determination of total plasminogen activator antigen in mouse plasma.

Background: Urokinase plasminogen activator is a serine protease that activates plasminogene to plasmin in the blood fibrinolytic system. It is also implicated in events related to cell invasion/migration

Assay Principle: Mouse uPA will bind to the capture antibody coated on the microtiter plate. Free, latent, and complexed enzyme will react with the capture antibody on the plate. After appropriate washing steps, polyclonal anti-Mouse uPA 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. 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 uPA

Reagents Provided:

♦Coated plate:
1-96 well immulon plate (8X12 removable wells) coated, blocked, and dried with uPA capture antibody
♦10X Wash Buffer:
1 bottle of 50ml wash; bring to 1X using DI water
♦Mouse uPA activity standard:
1 vial lyophilized standard
♦Anti-Mouse uPA primary antibody:
1 vial lyophilized polyclonal anti-mouse antibody
ASSAY PRINCIPLE
♦Anti-rabbit horseradish peroxidase conjugate secondary antibody:
1 vial concentrated HRP labeled antibody
♦TMB substrate solution: 10 ml

Storage and Stability: All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°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 4°C. Kit should be used no later than the expiration date.

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Reagents and Equipment Required:

- 1-channel pipettes covering 20-200µl, 500-5000µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 1.5ml centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- Blocking buffer
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Warnings:

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:

- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour 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 Reagents:

- TBS buffer: 0.10M TRIS, 0.15M NaCl, pH 7.4
- Blocking buffer (BSA): 3% BSA in TBS buffer

Specimen Collection:

Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate, preferably using Stabilyte™ evacuated vials. Immediately after collection of blood, samples must be centrifuged at 3000Xg for 15 minutes. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to anaylsis.

The uPA activity samples collected in the Stabilyte media are stable for up to 24 hours or stored at -20°C for up to one month and thawed three times without loss of uPA activity.

The assay measures total uPA in the 0.025-10 ng/ml range. Samples giving uPA levels above 10ng/ml should be diluted in plasma devoid of active uPA.

Assay Procedure:

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

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Preparation of the Standard: Dilution table for preparation of mouse uPA standards:

Mouse uPA: 100ng/ml before reconstitution

uPA concentration (ng/ml)	Dilutions
10	900µl (BSA) + 100µl (100ng/ml)
5	500µl (BSA) + 500µl (10ng/ml)
2	600µl (BSA) + 400µl (5ng/ml)
1	500µl (BSA) + 500µl (2ng/ml)
0.5	500µl (BSA) + 500µl (1ng/ml)
0.25	500µl (BSA) + 500µl (0.5ng/ml)
0.1	600µl (BSA) + 400µl (0.25ng/ml)
0.05	500µl (BSA) + 500µl (0.1ng/ml)
0.025	500µl (BSA) + 500µl (0.05ng/ml)
0	500µl (BSA) 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: Remove microtiter plate from bag. Add 100µl 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.

Primary Antibody Addition: Add 10ml 3% BSA blocking buffer directly to the primary antibody 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 Reagent Addition: Dilute 1µl into 10ml of 3% BSA 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. Quench the reaction by the addition of 50µl of 1M H_2SO_4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher

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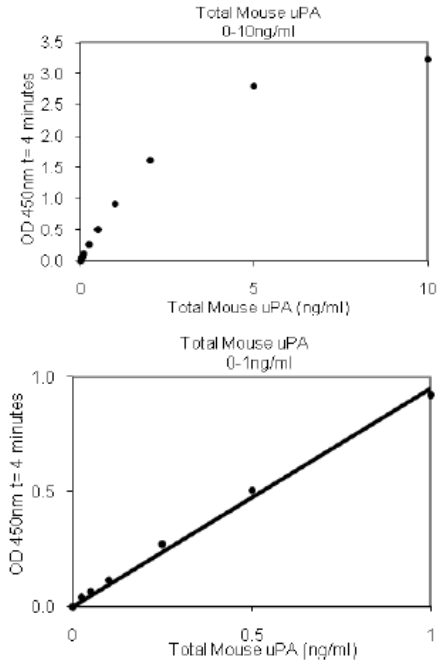
Measurement: Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration: Plot A_{450} against the amount of uPA in the standards. Fit a straight line through the points using a linear fit procedure. The uPA activity in the unknowns can be determined by from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)



Expected Values: The concentration level of uPA antigen in Mouse urine has been reported to be 1.8+/-1.9 ug/ml.

Abnormalities in uPA levels have been reported in the following condition:
♦ Venous Thrombosis: Low levels of uPA is associated with clot formation.
♦ Inflammatory Disease: Low levels of uPA may aggravate this condition.

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Performance Characteristics:

Linearity
The slope = 1.065
Correlation coefficient = 0.9992

Intra Assay Precision
High 3.1%, Medium 5.8%, Low 5.5%
(calculated by running 20 reps of each concentration in an assay).

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 or from contact with the above product.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THEREAPEUTIC USE.

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