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# Human Plasminogen Total Antigen ELISA Kit

**Catalog No:** CSI19819A **Lot No:** tbd **Size:** 1 Plate (1x 96 tests) **Expiration Date:** 1 year from receipt

| | |
|---|---|
| **Specificity:** | Total Human Plasminogen, plasmin and plasmin/antiplasmin complex |
| **Sensitivity:** | 0.087 ng/mL minimum detectable dose (MDD) |
| **Range:** | 0.2-200 ng/mL |
| **Sample Type:** | Plasma, serum, urine, cell culture media, tissue extracts. |

**Background:**

Plasminogen is a single chain glycoprotein zymogen and is the precursor of the fibrinolytic enzyme plasmin. Plasminogen deficiencies are classified as hypoplasminogenemia (Type I) or dysplasminogenemia (Type 2) and are associated with decreased extracellular fibrin clearance leading to mucous membrane lesions and ligneous conjunctivitis.

**Assay Principle:**

Human plasminogen, plasmin, and plasmin in complex with anti-plasmin in test samples and standards binds to the capture antibody that was coated on the microtiter plate during manufacture. After appropriate washing steps, polyclonal anti-human plasminogen primary antibody binds to the captured plasminogen. 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 substrate is used for color development at 450 nm. The amount of color development is directly proportional to the concentration of total plasminogen in the sample.

**Reagents Provided:**

| Description | Quantity |
|---|---|
| **CSI19819A – P**. 96-well microtiter strip plate coated with anti-human plasminogen antibody, dried and blocked on well surface | 1 plate: 96 wells (12 strips x 8 wells) |
| **CSI19819A - A.** Wash Buffer Concentrate (10x) | 1 bottle, 50 mL |
| **CSI19819A - B.** Human Plasminogen activity standard, lyophilized, **2000 ng/vial** | 1 vial |
| **CSI19819A - C.** Anti-plasminogen primary antibody, lyophilized. | 1 vial |
| **CSI19819A - D.** Anti-sheep horseradish peroxidase secondary antibody, concentrated | 1 vial |
| **CSI19819A - E**. TMB substrate solution | 1 bottle, 10 ml |



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**Storage and Stability:**

- Kit components should be stored at 4 °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 4 °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 $H_2SO_4$ or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

**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.
- All kit components must be kept refrigerated (4°C).
- **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.



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**Sample Collection:**

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Assay Procedure:**

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

**Preparation of Standard:**

Reconstitute standard as by adding **2 mL of Blocking Buffer (BB)** directly to the vial and agitate gently to completely dissolve contents. This will result in a **1,000 ng/mL** standard solution.

Prepare dilution series as indicated in the dilution table (at right) for preparation of human plasminogen standard.

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

| Plasminogen concentration (ng/ml) | Dilutions |
|---|---|
| 200 | 800µl (BB) + 200µl (from vial) |
| 100 | 500µl (BB) + 500µl (200ng/ml) |
| 50 | 500µl (BB) + 500µl (100ng/ml) |
| 20 | 600µl (BB) + 400µl (50ng/ml) |
| 10 | 500µl (BB) + 500µl (20ng/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) |
| 0.2 | 600µl (BB) + 400µl (0.5ng/ml) |
| 0 | 500µl (BB) Zero point to determine background |

**Standard and Unknown Addition:**

Remove microtiter plate from bag and add 100 µL plasminogen standards (in duplicate) and unknowns to wells. Record the position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

NOTE: The assay measures plasminogen and plasmin antigens in the 0.2-200 ng/mL range. If the unknown is thought to have high plasminogen/plasmin levels, dilutions may be made in a similar biological fluid devoid of plasminogen, or in blocking buffer. A 1:20,000 - 1:80,000 dilution for normal human plasma is suggested.

**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 µL to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.



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**Secondary Antibody Addition:**

Briefly centrifuge vial before opening. Dilute **1 µL of conjugated secondary antibody into 10 mL Blocking Buffer (BB)** and add 100 µL to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

**Substrate Incubation:**

Add 100 µL 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 µL 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.

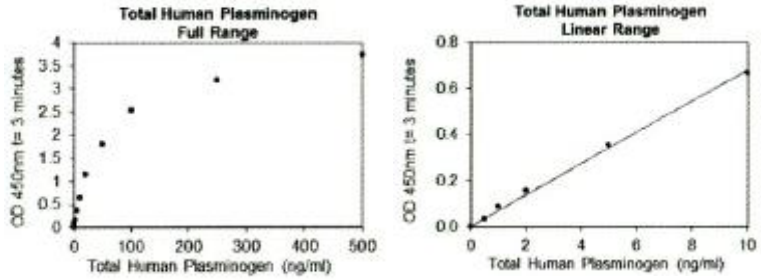
**Measurement:**

Set the absorbance at 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 plasminogen 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.

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




The amount of plasminogen in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

**Expected Values:**

The concentration of plasminogen was found to be 77-168 µg/mL in pooled donor plasma from normal individuals.

**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 $OD_{450}$: 0.076-0.094) and calculating the corresponding concentration. The MDD was 0.087 ng/mL.



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**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) | 1.54 | 5.05 | 42.3 |
| Standard Deviation | 0.091 | 0.265 | 3.93 |
| CV (%) | 5.91 | 5.25 | 9.28 |

**Performance Characteristics (continued):**

**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) | 5.63 | 6.39 | 50.6 |
| Standard Deviation | 0.563 | 0.805 | 3.79 |
| CV (%) | 10.0 | 12.6 | 7.49 |

**Recovery:** The recovery of antigen spiked to levels throughout the range of the assay in diluted plasma was evaluated.

| Sample | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| n | 4 | 4 | 4 | 4 |
| Mean (ng/ml) | 0.73 | 15.5 | 59.1 | 152 |
| Average % Recovery | 97 | 103 | 98 | 101 |
| Range | 80-120% | 96-112% | 87-112% | 97-106% |

**Linearity:** To assess the linearity of the assay, pooled citrated human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

| Sample | 1:2 | 1:4 | 1:8 | 1:16 |
|---|---|---|---|---|
| n | 4 | 4 | 4 | 4 |
| Average % of Expected | 99 | 106 | 102 | 120 |
| Range | 98-100% | 100-109% | 93-106% | 114-125% |

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

| Sample Type | Dilution | Mean (µg/ml) |
|---|---|---|
| Citrate Plasma | 1:80,000 | 98 |
| | 1:40,000 | 95 |



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**Specificity:** This assay recognizes natural human plasminogen, plasmin, and plasmin/antiplasmin complex. The factors listed below are prepared 250 ng/mL in buffer and assayed for cross-reactivity. No significant cross-reactivity was observed for the following:

Native mouse plasminogen
Native rat plasminogen
Native rabbit plasminogen
Native dog plasminogen
Native chicken plasminogen
Native bovine plasminogen

**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 | 0.2 ng/ml | 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 | 200 ng/ml | |
| B | 0 | 0.2 ng/ml | 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 | 200 ng/ml | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

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



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