

### INTENDED USE

This human plasminogen total assay is for the quantitative determination of total plasminogen and plasmin in human plasma, serum, urine, cell culture media, or tissue extracts. **For research use only.**

### BACKGROUND

Plasminogen is a single chain glycoprotein zymogen and is the precursor of the fibrinolytic enzyme plasmin. Plasminogen deficiencies are classified as hypoplasminogenemia (Type 1) or dysplasminogenemia (Type 2) and are associated with decreased extracellular fibrin clearance leading to mucous membrane lesions and ligneous conjunctivitis [1].

### ASSAY PRINCIPLE

Human plasminogen will bind to the capture antibody coated on the microtiter plate. Plasminogen, plasmin, and plasmin in complex with antiplasmin will react with the antibody on the plate. After appropriate washing steps, polyclonal anti-human plasminogen primary antibody binds to the captured protein. 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 450nm. The amount of color development is directly proportional to the concentration of total plasminogen in the sample.

### REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human plasminogen antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human plasminogen standard:** 1 vial lyophilized standard
- **Anti-human plasminogen primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-sheep 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<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. 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 plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . 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 by adding 2ml 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 human plasminogen standard:

Plasminogen concentration (ng/ml)	Dilutions
500	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (from vial)
250	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (500ng/ml)
100	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (250ng/ml)
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

**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 and add 100 $\mu\text{l}$  plasminogen 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.

NOTE: The assay measures plasminogen and plasmin antigens in the 0.5-500 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 dilution for normal human plasma is suggested for best results.

### Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. 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.

### Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 1 $\mu\text{l}$  of conjugated secondary antibody in 20ml of blocking buffer 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.

### Substrate Incubation

Add 100 $\mu\text{l}$  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 $\mu\text{l}$  of 1N  $\text{H}_2\text{SO}_4$  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.

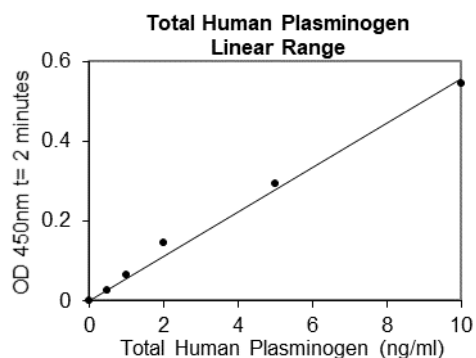
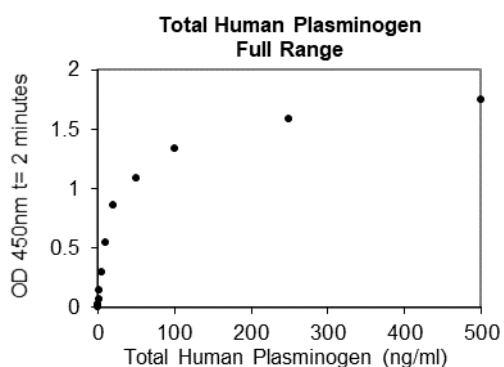
### Measurement

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 plasminogen 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 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.

A typical standard curve (EXAMPLE ONLY):



## EXPECTED VALUES

The concentration of plasminogen in pooled donor plasma from normal individuals was found to be  $195 \pm 10 \mu\text{g/ml}$  [2].

## 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  $OD_{450}$ : 0.077-0.087) and calculating the corresponding concentration. The MDD was 0.103 ng/ml.

**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

**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%

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

- Natural mouse plasminogen
- Natural rat plasminogen
- Natural rabbit plasminogen
- Natural dog plasminogen
- Natural chicken plasminogen
- Natural 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	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 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	250 ng/ml	500 ng/ml	
C												
D												
E												
F												
G												
H												

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

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:10,000	183
	1:20,000	211
EDTA Plasma	1:10,000	175
	1:20,000	169
Heparin Plasma	1:10,000	185
	1:20,000	194

**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**

1. Tefs K, *et al.*: Blood. 2006, 108(9):3021-26.
2. Zolton RP, *et al.*: Clin Chem. 1972,18:654-7.