Catalog # HPLGKT-TOT

Human Plasminogen Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This human plasminogen total assay is for the quantitative determination of total plasminogen and plasmin in biological fluids.

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 [1].

ASSAY PRINCIPLE

Human plasminogen will bind to the capture antibody provided for coating 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 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 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 strips 8x12):** containing anti-human plasminogen antibody dried and blocked on the strip well surface

♦**10X Wash Buffer:**
1 bottle of 50ml; bring to 1X using DI water

♦**Human Plasminogen activity standard:**
1 vial lyophilized standard

♦**Anti-plasminogen primary antibody:**
1 vial lyophilized polyclonal sheep anti-mouse antibody

♦**Anti-sheep horseradish peroxidase secondary antibody:**
1 vial concentrated HRP labeled antibody

♦**TMB substrate solution:**
1 bottle 10ml solution

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.

REAGENTS AND EQUIPMENT REQUIRED

- Pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes

- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers
- ELISA plate cover
- TBS buffer
- 3% BSA 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.
- All kit components must be stored at the correct temperatures.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris-HCl 0.15M NaCl, pH 7.4
- **Blocking buffer (BSA):** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Samples of human plasma, serum, urine, cell culture media, or tissue extracts may be applied directly to the plate.

The assay measures total plasminogen in the 0-500 ng/ml range. Samples giving plasminogen levels above 200 ng/ml should be diluted in a similar biological fluid devoid of plasminogen. Blocking buffer may also be used. A dilution of at least 1:10,000 is recommended for measurement of plasminogen in normal human plasma.

ASSAY PROCEDURE

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

Preparation of Standard:

Reconstitute standard as directed on vial to give a 1,000 ng/mL standard solution.

Dilution table for preparation of human plasminogen standards:

Plasminogen concentration (ng/ml)	Dilutions
500	500µl (BSA) + 500µl (1,000ng/ml)
250	500µl (BSA) + 500µl (500 ng/ml)
100	600µl (BSA) + 400µl (250ng/ml)
50	500µl (BSA) + 500µl (100ng/ml)
20	600µl (BSA) + 400µl (50ng/ml)
10	500µl (BSA) + 500µl (20ng/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	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:
Add 100µl of 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 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.
NOTE: If the unknown is thought to have high plasminogen levels, dilutions may be made in plasma devoid of plasminogen, or in blocking buffer. A 1:10,000 dilution, generated by two serial dilutions of 1:100 each, is recommended for the measurement of plasminogen in normal human plasma.

Primary Antibody Addition:
Reconstitute primary antibody as directed on 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.

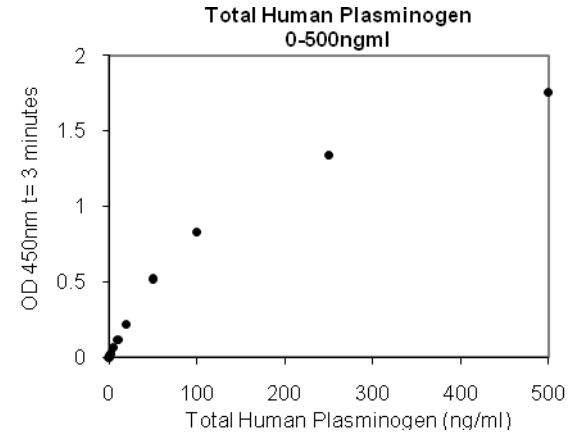
Secondary Antibody Addition:
Dilute 1µl into 10ml blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

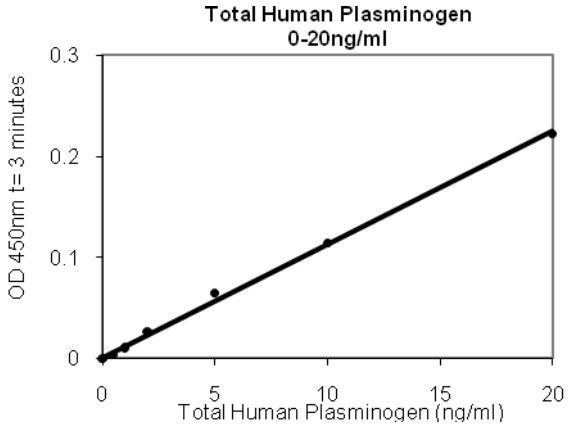
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450nm. For best results read plate immediately.

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 plasminogen in the standards. Fit a straight line through the points using a linear fit procedure. The plasminogen in the unknowns can be determined from this curve.

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





EXPECTED VALUES

The concentration of plasminogen in pooled donor plasma from normal individuals was found to be 195 ± 10 µg/ml [2].

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.

REFERENCES

1. Tefs K, *et al.*: Molecular and clinical spectrum of type I plasminogen deficiency: A series of 50 patients. Blood, Nov 2006; 108(9): 3021 - 3026.

2. Zolton RP, *et al.*: Assay of Human Plasminogen in Plasma by Affinity Chromatography. Clin. Chem., Jul 1972; 18: 654 - 657.

Example of ELISA Kit Plate Layout
96 Well Plate
Standards: 22 Wells
Samples: 74 Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	250ng/ml	500ng/ml	
B	0	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	250ng/ml	500ng/ml	
C												
D												
E												
F												
G												
H												