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

- Immunoassay plate:
  1-96 well immulon plate
- Anti-human plasminogen capture antibody:
  1 vial of frozen monoclonal antibody
- Plate blocking reagent:
  1 bottle of 30 ml blocking buffer
- 10X wash buffer:
  1 bottle of 50 ml wash; bring to 1X using DI water
- Human plasminogen standard:
  1 vial of frozen standard
- Anti-human plasminogen primary antibody:
  1 vial of frozen polyclonal antibody
- Anti-sheep horseradish peroxidase secondary antibody:
  1 vial of concentrated HRP labeled antibody
- TMB substrate solution:
  1 bottle of 10 ml solution

STORAGE AND STABILITY

Frozen components must be stored at -20°C or colder when not in use. All other reagents 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:** 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 0.5-200 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.

PLATE PREPARATION

Dilute human plasminogen capture antibody by adding 10µl antibody to 10ml of TBS. Add 100µl of diluted antibody to each well of plate. Cover plate and incubate at room temperature overnight. Aspirate plate and wash wells three times with 300µl wash buffer. Add 300µl of the included plate blocking reagent to all wells. Incubate at room temperature for at least 30 minutes. Aspirate plate and remove excess buffer by gently tapping plate on paper towel or kimwipe.

ASSAY PROCEDURE

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

Preparation of Standard:
Dilution table for preparation of human plasminogen standards:

Human Plasminogen Concentration:
7.2mg/mL = 7,200,000ng/mL

1) Make an intermediate of 72,000ng/mL= 247.5µL (BSA) + 2.5µL (Human Plasminogen Standard Stock)

2) Use intermediate (1) to make an intermediate (2) of 720ng/mL= 495µL (BSA) + 5µL (intermediate 1)

3) Use intermediate (2) to continue making the standards in the dilution table below.

Plasminogen concentration (ng/ml)	Dilutions
200	722µl (BSA) + 278µl (intermediate 2)
100	500µl (BSA) + 500µl (200ng/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)

NOTE: DILUTIONS FOR THE STANDARD CURVE 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:

Dilute 20µl 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.

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. Quench the reaction with the addition of 50µl of 1N H_2SO_4 and read final absorbance values at 450nm.

NOTE: Time for substrate development is dependent on needs of researcher.

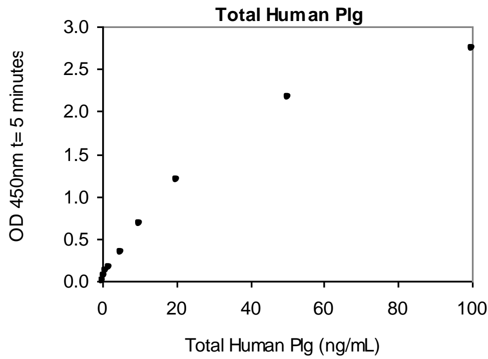
Measurement:

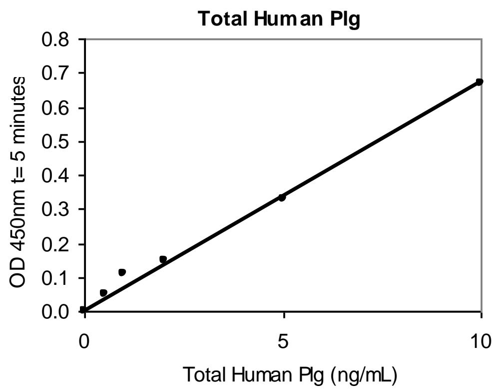
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A_{450}.

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.