Human Plasminogen Total Antigen ELISA

For Research Use Only

INTRODUCTION

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

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).

PRINCIPLES OF PROCEDURE

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 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.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Anti-human plasminogen coated 96-well plate	1 plate	4°C	PI97a
Standard	Human plasminogen activity standard	1 vial	4°C	PI97b
Primary Antibody	Sheep anti-human plasminogen antibody	1 vial	4°C	PI97c
Wash Buffer	10x solution for washing plate	50 mL	4°C	PI97d
Substrate	TMB Substrate	10 mL	4°C	PI97e
Secondary Antibody	Anti-sheep HRP conjugated antibody	1 vial	4°C	PI97f

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μl and 200-1000 μl tips
2. 12-channel pipette covering 30-300μl
3. 1N H_2SO_4
4. DI water
5. Microtiter plate spectrophotometer with a 450 nm filter
6. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. 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.

PROCEDURAL NOTES

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

SAMPLE COLLECTION AND PREPARATION

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 500 ng/ml should be diluted in a similar biological fluid devoid of plasminogen or 3% BSA Blocking Buffer. A dilution of 1:1,000 to 1:10,000 is recommended for the measurement of plasminogen in normal human plasma.

REAGENT PREPARATION

1. Dilute the 50 mL of 10x Wash Buffer concentrate to 1x with 450 mL of DI water prior to use.
2. Prepare 100 mL of TBS Buffer: 0.1 M Tris-HCL, 0.15 M NaCl, pH 7.4
3. Prepare 20 mL of 3% Blocking Buffer: 3% BSA in TBS Buffer.

STANDARD PREPARATION

Reconstitute the Standard as directed on the vial to give a 1,000 ng/mL Standard Stock Solution. **Do not prepare the Standards until you are ready to apply them to the plate.**

Table 1: Preparation of Standard Curve

Standard	Plasminogen Concentration (ng/mL)	Blocking Buffer (μL)	Transfer Volume (μL)	Transfer Source	Final Volume (μL)
S_{10}	500	500	500	Stock Vial	500
S_9	250	500	500	S_{10}	600
S_8	100	600	400	S_9	500
S_7	50	500	500	S_8	600
S_6	20	600	400	S_7	500
S_5	10	500	500	S_6	500
S_4	5	500	500	S_5	600
S_3	2	600	400	S_4	500
S_2	1	500	500	S_3	500
S_1	0.5	500	500	S_2	1000

ASSAY PROCEDURE

1. Add 100 μl of the Standards and unknowns to wells in duplicate. If the unknown is thought to have high plasminogen levels, dilutions may be made in plasma devoid of plasminogen, or in 3% BSA 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. For a suggested plate layout, see Scheme I below.
2. Shake the plate at 300 rpm for 30 minutes at room temperature.
3. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
4. Reconstitute the Primary Antibody as directed on the vial and agitate gently to completely dissolve contents. Add 100 µl to each well.
5. Shake the plate at 300 rpm for 30 minutes at room temperature.
6. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
7. Dilute the Secondary Antibody in 3% BSA Blocking Buffer as directed on the vial and add 100 µl to each well.
8. Shake the plate at 300 rpm for 30 minutes at room temperature.
9. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
10. Add 100 μl of TMB Substrate to each well.
11. Shake the plate at 300 rpm for 5-15 minutes at room temperature.
12. Stop the reaction with 50 μl of 1N H_2SO_4, and read the plate at 450 nm.

Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_{10}	S_{10}	S_2	S_2	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}
B	S_9	S_9	S_1	S_1	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}
C	S_8	S_8	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}
D	S_7	S_7	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}
E	S_6	S_6	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
F	S_5	S_5	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
G	S_4	S_4	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
H	S_3	S_3	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}

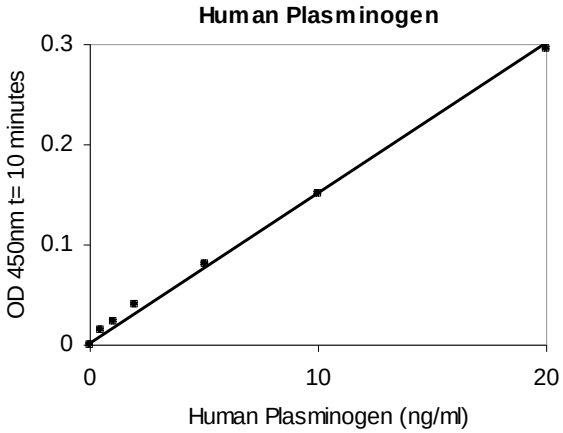
EXPECTED VALUES

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

CALCULATIONS

1. Plot the A_{450} against the concentration of plasminogen in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the plasminogen concentrations in the unknowns using the standard curve. See Figure 1 on the following page for an example of a typical standard curve.

Figure 1: Typical Standard Curve
(Do Not Use For Calculations)



REFERENCES

1. Tefs, K., *et al*.; (2006) Blood; 108(9): 3021-3026
2. Zolton, R.P., *et al*.; (1972) Clin. Chem.;18: 654-657

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