

Enzyme Immunoassay for Mouse Plasminogen Total Antigen

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INTRODUCTION

This mouse 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 1) or dysplasminogenemia (Type 2) and are associated with decreased extracellular fibrin clearance leading to mucous membrane lesions and ligneous conjunctivitis (1).

PRINCIPLES OF PROCEDURE

Mouse 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-mouse 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-mouse plasminogen coated 96-well plate	1 plate	4°C	PI96a
Standard	Mouse plasminogen activity standard	1 vial	4°C	PI96b
Primary Antibody	Rabbit anti-mouse plasminogen antibody	1 vial	4°C	PI96c
Wash Buffer	10x solution for washing plate	50 mL	4°C	PI96d
Substrate	TMB substrate	10 mL	4°C	PI96e
Secondary Antibody	Anti-rabbit HRP conjugated antibody	1 vial	4°C	PI96f

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μ l and 200-1000 μ l and tips
2. 12-channel pipette covering 30-300 μ l
3. 1N H₂SO₄
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.
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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.
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SAMPLE COLLECTION AND PREPARATION

Samples of mouse 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 mouse 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% BSA Blocking Buffer: 3% BSA in TBS Buffer.
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STANDARD PREPARATION

Reconstitute the standard as directed on the vial to give a 500 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 ₈	500	0	100	Stock Vial	500
S ₇	250	500	500	S ₈	600
S ₆	100	600	400	S ₇	500
S ₅	50	500	500	S ₆	600
S ₄	20	600	400	S ₅	500
S ₃	10	500	500	S ₄	500
S ₂	5	500	500	S ₃	500
S ₁	2.5	500	500	S ₂	1,000

ASSAY PROCEDURE

1. Add 100 μ l of the Standards and unknowns to the 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 mouse 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 μ L 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 μ l to each well.
5. Shake the plate at 300 rpm for 30 minutes at room temperature.
6. Wash the plate three times with 300 μ L 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 μ l to each well.
8. Shake the plate at 300 rpm for 30 minutes at room temperature.
9. Wash the plate three times with 300 μ L of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
10. Add 100 μ l 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 μ l of 1N H₂SO₄ and read the plate at 450 nm.

Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S8	S8	U1	U1	U9	U9	U17	U17	U25	U25	U33	U33
B	S7	S7	U2	U2	U10	U10	U18	U18	U26	U26	U34	U34
C	S6	S6	U3	U3	U11	U11	U19	U19	U27	U27	U35	U35
D	S5	S5	U4	U4	U12	U12	U20	U20	U28	U28	U36	U36
E	S4	S4	U5	U5	U13	U13	U21	U21	U29	U29	U37	U37
F	S3	S3	U6	U6	U14	U14	U22	U22	U30	U30	U38	U38
G	S2	S2	U7	U7	U15	U15	U23	U23	U31	U31	U39	U39
H	S1	S1	U8	U8	U16	U16	U24	U24	U32	U32	U40	U40

EXPECTED VALUES

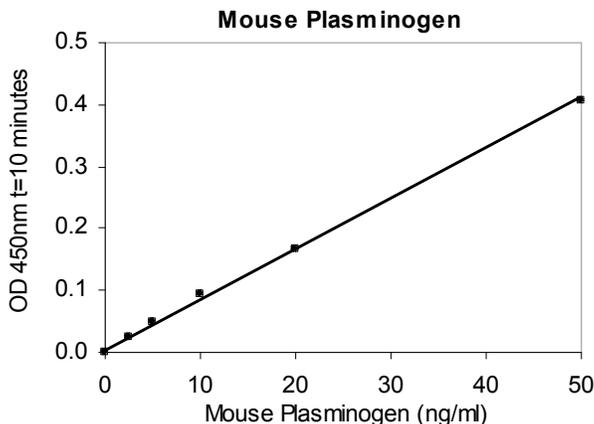
The concentration of human plasminogen in pooled donor plasma from normal individuals was found to be 195 ± 10 μ g/ml (2). Normal values of plasminogen in mouse plasma have not been conclusively determined but are believed to be similar to human plasma. Plasminogen antigen was found to be 84 ± 8 μ g/ml in a small sample (n=4) of normal mice (3). Hypercoagulable states may be induced by genetically decreased plasminogen (4) or abnormal plasminogen (5).

CALCULATIONS

1. Plot the A₄₅₀ 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

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