

Mouse Plasmin Activity Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This mouse plasmin activity assay is for the quantitative determination of active plasmin in mouse plasma, other biological fluids, and cell culture media.

BACKGROUND

Plasmin is an enzyme present in blood that degrades many proteins found in blood plasma, most notably fibrin clots. Plasmin is a serine protease that is released as the zymogen plasminogen into the circulation and activated by tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), thrombin, fibrin, and factor XII (Hageman factor). Active plasmin in plasma is rapidly inactivated by forming a covalent complex with the serine protease inhibitor (serpin) alpha 2-antiplasmin. Decreased levels of plasmin may lead to thrombosis due to inadequate ability to dissolve clots.

ASSAY PRINCIPLE

Functionally active plasmin in samples will bind to biotinylated active site inhibitor peptide in solution on the reaction plate. Only free active enzyme will be inhibited and not plasminogen or complexed plasmin. Inhibited plasmin will bind to capture antibody coated on the microtiter plate. After appropriate washing steps, biotinylated plasmin is reacted with avidin conjugated to horseradish peroxidase. TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of plasmin.

REAGENTS PROVIDED

- ◆ **Non-binding reaction plate:**
1-96 well plate (8X12 well removable strips) with non-binding surface
- ◆ **Immunoassay plate:**
1-96 well immulon plate (8X12 removable strips) coated with capture antibody, blocked and dried
- ◆ **10X Wash Buffer:**
1 bottle of 50ml wash; bring to 1X using DI water
- ◆ **Mouse plasmin activity standard:**
1 vial of lyophilized standard
- ◆ **Biotinylated inhibitor**
1 vial of lyophilized biotinylated inhibitor
- ◆ **Avidin peroxidase:**
1 vial of concentrated HRP labeled avidin
- ◆ **TMB substrate solution:**
1 bottle of 10 ml solution

STORAGE AND STABILITY

Kit components should be stored at 4°C when not in use. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H₂SO₄
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- 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 kept refrigerated (4°C).

•**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 plasma, serum, cell culture media, or other biological fluids may be used directly in the assay. The assay measures active mouse plasmin in the 0.02-10 µg/ml range. Samples giving plasmin levels above 10 µg/ml should be diluted in BSA before use.

ASSAY PROCEDURE

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

Preparation of Standard:

Reconstitute standard as directed on vial to give a **25µg/ml** standard stock solution. Prepare the plasmin standard curve according to the following dilution table.

Plasmin concentration (µg/ml)	Dilutions
10	600µl (BSA) + 400µl (25µg/ml)
5	500µl (BSA) + 500µl (10µg/ml)
2	600µl (BSA) + 400µl (5µg/ml)
1	500µl (BSA) + 500µl (2µg/ml)
0.5	500µl (BSA) + 500µl (1µg/ml)
0.2	600µl (BSA) + 400µl (0.5µg/ml)
0.1	500µl (BSA) + 500µl (0.2µg/ml)
0.05	500µl (BSA) + 500µl (0.1µg/ml)
0.02	600µl (BSA) + 400µl (0.05µg/ml)
0	500µl (BSA) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD AND ZERO STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:

Reconstitute biotinylated inhibitor as directed on vial and add 20µl to each well of the non-binding reaction plate. Add 100µl of plasmin standards and unknowns in duplicate to wells. Carefully record their position. Incubate plate at room temperature for 30 minutes.

DO NOT WASH PLATE.

Add 100µl solution from each well to the corresponding wells of the immunoassay plate. 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.

Avidin Peroxidase Addition:

Dilute 2µl of avidin peroxidase into 10ml of 3% BSA 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 H₂SO₄ 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:

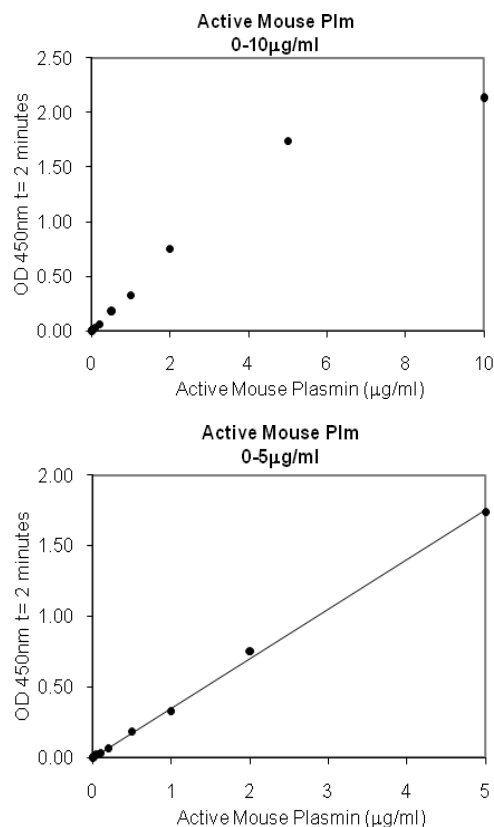
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:

Plot A_{450} against the amount of plasmin in the standards. Fit a straight line through the points using a linear fit procedure. The active plasmin concentration in the unknowns can be determined from this curve.

A typical standard curve.

(EXAMPLE ONLY, DO NOT USE)



EXPECTED VALUES

Free active plasmin concentrations in plasma are low except in cases of antiplasmin deficiency [1] or thrombolytic therapy [2]. In house testing of pooled normal mouse plasma in citrate indicates an active plasmin concentration of 0.2 µg/ml. Plasminogen antigen was found to be 84 ± 8 µg/ml in a small sample (n=4) of normal mice [3]. Hypercoagulable states may be induced by decreased levels of plasmin caused by decreased plasminogen [4] or abnormal plasminogen [5].

PERFORMANCE CHARACTERISTICS

The assay measures active plasmin in the 0.02-10 µg/ml range. Dilutions of samples should be performed to assure the unknowns will be in the assay range.

DISCLAIMER

This information is believed to be correct but does not purport 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. Carpenter, SL & P Mathew: Alpha2-antiplasmin and its deficiency: fibrinolysis out of balance. *Haemophilia*, Nov 2008; 14(6): 1250-4.
2. Stief, TW *et al.*: Monitoring of Plasmin and Plasminogen Activator Activity in Blood of Patients under Fibrinolytic

Treatment by Reteplase. *Clinical & Applied Thrombosis/ Hemostasis*, Apr 2006; 12: 213 - 218.

3. Ploplis VA, *et al.*: Effects of Disruption of the Plasminogen Gene on Thrombosis, Growth, and Health in Mice. *Circulation*, Nov 1995; 92: 2585 - 2593.

4. Lottenberg R, *et al.*: Recurring thromboembolic disease and pulmonary hypertension associated with severe hypoplasminogenemia. *Am J Hematol*. 1985; 19:181-93.

5. Aoki N, *et al.*: Abnormal plasminogen. A hereditary molecular abnormality found in a patient with recurrent thrombosis. *J Clin Invest*. 1978; 61:1186-95.

Layout Example of 96 Well Plate

Standards: 20 wells

Samples: 76 wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.02µg/ml	0.05µg/ml	0.1µg/ml	0.2µg/ml	0.5µg/ml	1µg/ml	2µg/ml	5µg/ml	10µg/ml		
B	0	0.02µg/ml	0.05µg/ml	0.1µg/ml	0.2µg/ml	0.5µg/ml	1µg/ml	2µg/ml	5µg/ml	10µg/ml		
C												
D												
E												
F												
G												
H												