Mouse Plasminogen ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Add 50 µl of standard/samples per well.
Incubate 2 hours.

Wash, then add 50 µl of
biotinylated antibody per well.
Incubate 1 hour.

Wash, then add 50 µl of SP per well.
Incubate 30 minutes.

Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 10 minutes.

Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax Mouse Plasminogen ELISA Kit

Catalog No. EMP1200-1
Sample Insert/Reference Only

Introduction

Plasminogen is a single chain glycoprotein zymogen that is synthesized in the liver and circulated in plasma with a molecular weight of 90 kDa. The N-terminal portion of the molecule is made up of five kringle domains that bind to fibrin. The native molecule has an amino-terminal glutamic acid, known as glu-plasminogen, but this can undergo proteolytic cleavage by plasmin to lys-plasminogen (1). The inactive proenzyme plasminogen is converted to the active enzyme plasmin that ultimately digests fibrin. Tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) catalyzes the activation of plasminogen, while plasminogen activator inhibitors (PAIs) inhibits the activation (2). The plasminogen system plays a role in macrophage recruitment, arterial stenosis, atherosclerosis, aneurysm formation, skin and corneal wound healing, glomerulonephritis, and neovascularization (3).

Principle of the Assay

The AssayMax Mouse Plasminogen ELISA kit is designed for detection of mouse plasminogen in urine and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures plasminogen in less than 4 hours. A polyclonal antibody specific for plasminogen has been pre-coated onto a 96-well microplate with removable strips. Plasminogen in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for plasminogen, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**

- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Mouse Plasminogen Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against mouse plasminogen.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Mouse Plasminogen Standard:** Mouse plasminogen in a buffered protein base (96 ng, lyophilized).
- **Biotinylated Mouse Plasminogen Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against mouse plasminogen (80 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate)**: A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate and biotinylated antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8°C.

- Store standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3000 x *g* for 10 minutes to remove debris. Dilute cell culture media into EIA Diluent. The user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:200 with EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Standard Curve:** Reconstitute the 96 ng of Mouse Plasminogen Standard with 4 ml of EIA Diluent to produce 24 ng/ml of solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (24 ng/ml) 1:2 with equal volume of EIA Diluent to produce 12, 6, 3, 1.5, 0.75, 0.375, and 0.188 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Mouse Plasminogen] (ng/ml)
P1	1 part Standard (24 ng/ml) + 1 part EIA Diluent	12.00
P2	1 part P1 + 1 part EIA Diluent	6.000
P3	1 part P2 + 1 part EIA Diluent	3.000
P4	1 part P3 + 1 part EIA Diluent	1.500
P5	1 part P4 + 1 part EIA Diluent	0.750
P6	1 part P5 + 1 part EIA Diluent	0.375
P7	1 part P6 **+** 1 part EIA Diluent	0.188
P8	EIA Diluent	0.000

- **Biotinylated Mouse Plasminogen Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Mouse Plasminogen Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Mouse Plasminogen Antibody to each well and incubate for 1 hour.
- Wash a microplate as described above.

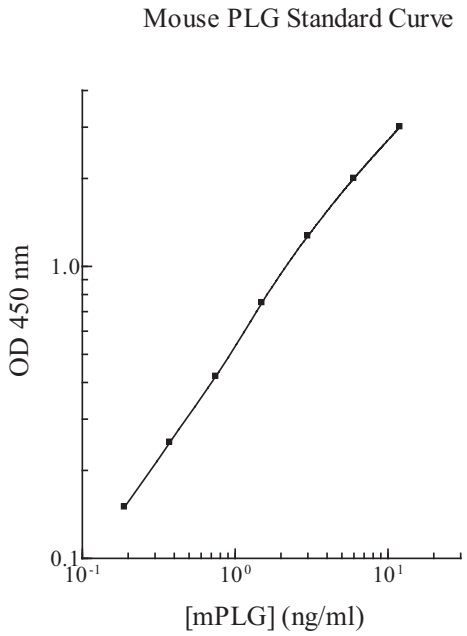
- Add 50 μl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash a microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of mouse plasminogen is typically ~ 0.18 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9 % and 7.1 % respectively.
- This assay recognizes both natural and recombinant mouse plasminogen.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1:100	91%
1:200	98%
1:400	103%

Recovery

Standard Added Value	1 - 10 ng/ml
Recovery %	86-117 %
Average Recovery %	98 %

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	None
Rat	<60%
Human	None
Swine	None
Rabbit	None
Mouse	100%

References

(1) Forsgren, M. *et al.* (1987) *FEBS Letters* 213:254
(2) Collen, D. and Lijnen, H.R. (1991) *Blood* 78:3114
(3) Carmeliet, P. and Collen, D. (1996) *Semin. Thromb. Hemost*. 22:525

Version 2.1R1

Related Products

- EMP2211-1 AssayMax Mouse Plasminogen ELISA Kit (Plasma and Serum samples)
- EBP2211-1 AssayMax Bovine Plasminogen ELISA Kit (Plasma and Serum samples)
- EBP1200-1 AssayMax Bovine Plasminogen ELISA Kit (Urine and Cell Culture Supernatant samples)
- EP1200-1 AssayMax Human Plasminogen ELISA Kit (Plasma, Urine, Saliva, Milk, and Cell Culture Supernatant samples)
- ERP1200-1 AssayMax Rat Plasminogen ELISA Kit (Plasma, Serum, Urine, and Cell Culture Supernatant samples)