Mouse Plasminogen ELISA Kit

Vertrieb:
L O X O GmbH Immunbiologie Biochemie, Produkte und Systeme
Postfach 11 30 69215 Dossenheim
Telefon +49 (0) 62 21 - 86 80 23 **FAX** +49 (0) 62 21 - 86 80 255
E-Mail: info@loxo.de **Internet:** www.loxo.de

Assaypro LLC
30 Triad South Drive
St. Charles, MO 63304
T (636) 447-9175
F (636) 447-9475
www.assaypro.com

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 25 µl of standard/samples
and 25 µl of biotinylated protein per well.
Incubate 2 hours.

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

Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 8 minutes.

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

Assay Template

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

AssayMax Mouse Plasminogen ELISA Kit

Catalog No. EMP2211-1
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 plasma and serum. This assay employs a quantitative competitive enzyme immunoassay technique that measures plasminogen in less than 3 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 competed with a biotinylated plasminogen sandwiched by the immobilized antibody and 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 protein, 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 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 (4 μg, lyophilized, 2 vials).
- **Biotinylated Mouse Plasminogen:** 1 vial, lyophilized.
- **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, 1 bottle).
- **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 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 and biotinylated protein 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

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute plasma 1:80 into 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. (EDTA or Heparin can also be used as an anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute serum 1:80 into 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 4 µg of Mouse Plasminogen Standard with 0.5 ml of EIA Diluent to produce 8 µg/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 (8 µg/ml) 1:2 with equal volume of EIA Diluent to produce 4, 2, 1, and 0.5 µg/ml solutions. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Mouse PLG] (µg/ml)
P1	Standard (8 µg/ml)	8.00
P2	1 part P1 + 1 part EIA Diluent	4.00
P3	1 part P2 + 1 part EIA Diluent	2.00
P4	1 part P3 + 1 part EIA Diluent	1.00
P5	1 part P4 + 1 part EIA Diluent	0.50
P6	EIA Diluent	0.00

- **Biotinylated Mouse Plasminogen (2x):** Dilute Biotinylated Mouse Plasminogen with 4 ml EIA Diluent to produce a 2-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:2 with EIA Diluent. Any remaining solution should be frozen at -20°C and used within 30 days..

- **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

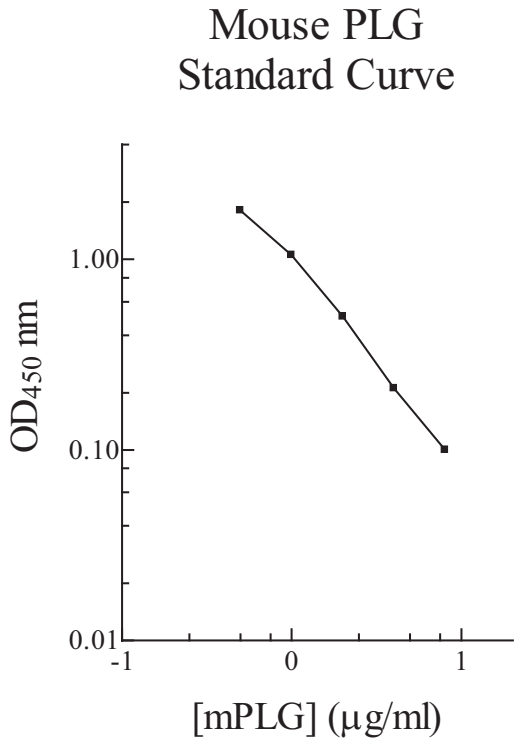
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of Mouse Plasminogen Standard and/or sample per well, and immediately add 25 µl of Biotinylated Mouse Plasminogen to each well (on top of the standard or sample). Cover wells with a sealing tape and incubate for 2 hours at room temperature. 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 Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 8 minutes or until the optimal blue color density develops. Gently tap 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 four-parameter or log-log 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 plasminogen is typically ~ 0.5 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7 % and 7.1 % respectively.
- This assay recognizes both natural and recombinant mouse plasminogen.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Mouse Plasma**	**Mouse Serum**
1:40	95%	92%
1:80	98%	96%
1:160	103%	104%

Recovery

Standard Added Value	0.5 – 5 µg/ml
Recovery %	84-113 %
Average Recovery %	98 %

Cross-Reactivity

Species	**% Cross Reactivity**
Canine	None
Bovine	None
Monkey	<2%
Rat	<5%
Human	None
Swine	<2%
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 1.2R1

Related Products

- EMP1200-1 AssayMax Mouse Plasminogen ELISA Kit (Urine and Cell Culture 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)