



Rat Plasminogen ELISA Kit

Vertrieb:

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

Thank You for choosing Assaypro

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.

Assay Summary

Add 50 μ l of Standard/ Sample per well.
Incubate 2 hours.



Wash, then add 50 μ l of
Biotinylated Antibody per well.
Incubate 1 hour.



Wash, then add 50 μ l of
SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 μ l of
Chromogen Substrate per well.
Incubate 12 minutes.



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

AssayMax Rat Plasminogen ELISA Kit

Catalog No. ERP1200-1
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) inhibit 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 Rat Plasminogen ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of rat plasminogen in plasma, serum, urine, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures rat plasminogen in less than 4 hours. A polyclonal antibody specific for rat 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 rat 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

- **Rat Plasminogen Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat plasminogen.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Rat Plasminogen Standard:** Rat plasminogen in a buffered protein base (800 ng, lyophilized).
- **Biotinylated Rat Plasminogen Antibody (70x):** A 70-fold concentrated biotinylated polyclonal antibody against rat plasminogen (105 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

- Upon arrival, immediately store components of the kit at recommended temperatures 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 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days 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 μ l, 20-200 μ l, 200-1000 μ l, 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 samples 1:20000 with EIA Diluent or within the range of 1:5000 – 1:50000. 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. Dilute samples 1:20000 with EIA Diluent or within the range of 1:5000 – 1:50000. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3000 x *g* for 10 minutes to remove debris. The user should determine the optimal dilution factor. Dilute cell culture media into EIA Diluent and assay. Store samples 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 urine 1:4 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 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 800 ng of Rat Plasminogen Standard with 2 ml of EIA Diluent to produce a 400 ng/ml standard 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 (400 ng/ml) 1:4 with EIA Diluent to produce 100, 25, 6.25, 1.56, and 0.391 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	[Rat Plasminogen] (ng/ml)
P1	Standard (400 ng/ml)	400.0
P2	1 part P1 + 3 parts EIA Diluent	100.0
P3	1 part P2 + 3 parts EIA Diluent	25.00
P4	1 part P3 + 3 parts EIA Diluent	6.250
P5	1 part P4 + 3 parts EIA Diluent	1.563
P6	1 part P5 + 3 parts EIA Diluent	0.391
P7	EIA Diluent	0.000

- **Biotinylated Rat Plasminogen Antibody (70x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:70 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, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°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 Rat Plasminogen Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last 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 Rat Plasminogen antibody to each well and incubate for 1 hour.
- Wash the 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 the microplate as described above.

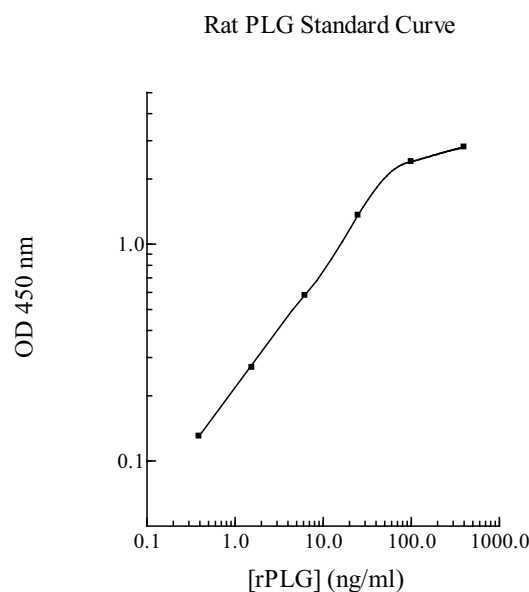
- Add 50 μ l of Chromogen Substrate per well and incubate for 12 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μ l of stop solution to each well. The color will change from blue to yellow.
- 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 rat plasminogen is typically ~ 0.3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.1% respectively.
- This assay recognizes both natural and recombinant rat plasminogen.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:10000	90%	91%
1:20000	97%	98%
1:40000	105%	103%

Sample Dilution	Average Percentage of Expected Value
	Urine
1:2	83%
1:4	97%
1:8	105%

Recovery

Standard Added Value	1.5 – 100 ng/ml
Recovery %	84 – 109%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Canine	<10%
Bovine	None
Monkey	<10%
Rat	100%
Human	<5%
Swine	<5%
Rabbit	None
Mouse	None

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

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