Plg (Rat) ELISA Kit

Catalog Number KA3831

96 assays

Version: 02

Intended for research use only

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Introduction

Background

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).

Principle of the Assay

The Plg (Rat) ELISA 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 washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Rat Plasminogen Microplate: A 96-well polystyrene microplate coated with a polyclonal antibody against rat plasminogen.	96 (8 x 12) wells
Sealing Tapes: Precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Rat Plasminogen Standard: Rat plasminogen in a buffered protein base (lyophilized).	800 ng
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 x 2
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 Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperature 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.

Materials Required but Not Supplied

- ✓ 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.

Precautions for Use

- ✓ This product is to be used solely for in vitro Research Use Only and is not to be used for diagnostic purposes.
- ✓ Prepare all reagents (working diluent buffer, wash buffer, standard, 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.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acidic solution.

Assay Procedure

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 stock 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 stock solution (400 ng/mL) 1:4 with EIA Diluent to produce 100, 25, 6.25, 1.56, and 0.391 ng/mL of 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	1 part 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.

Sample Preparation

- ✓ 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.

Assay Procedure

1. 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).
2. 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.
3. 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 sample addition.
4. 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.
5. Add 50 µL of Biotinylated Rat Plasminogen antibody to each well and incubate for 1 hour.
6. Wash the microplate as described above.
7. 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.
8. Wash the microplate as described above.
9. Add 50 µL 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.
10. Add 50 µL of stop solution to each well. The color will change from blue to yellow.
11. 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.

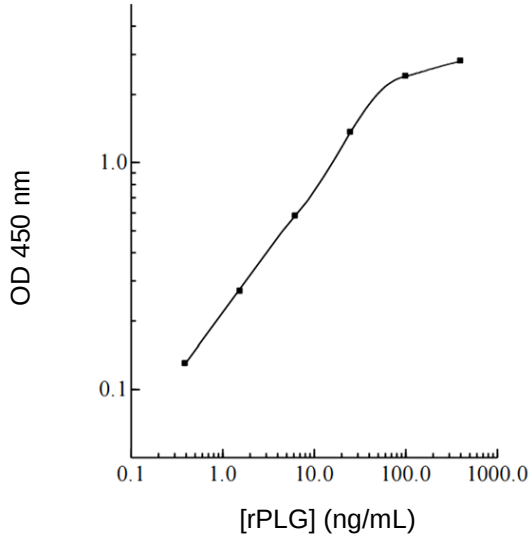
✓ Assay Summary

1. Add 50 µL of Standard or Sample per well. Incubate 2 hours.
2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour.
3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 12 minutes.
5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

- ✓ 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.



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 7.2 % respectively.
- ✓ This assay recognizes both natural and recombinant rat plasminogen.

- Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:10000	89%	95%
1:20000	98%	97%
1:40000	103%	101%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1.2	84%
1:4	99%
1:8	101%

- Recovery

Standard Added Value	2.5 - 100 ng/mL
Recovery %	87-117%
Average Recovery %	97.5%

- Cross-Reactivity

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

Resources

References

1. Forsgren, M. et al. (1987) FEBS Letters 213:254
2. Collen, D. and Lijnen, H.R. (1991) Blood 78:3114

Plate Layout

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