AssayMax Human Plasminogen ELISA Kit

Catalog No. EP1200-1

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).

Principal of the Assay

The AssayMax Human Plasminogen ELISA kit is designed for detection of human plasminogen in plasma, cell culture supernatants, and tissue samples. This assay employs a quantitative sandwich 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 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

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Plasminogen Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human plasminogen.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Plasminogen Standard:** Human plasminogen in a buffered protein base (320 ng, lyophilized).
- **Biotinylated Plasminogen Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against human plasminogen (80 µl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).

- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate)**: A 100-fold concentrate (90 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 kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

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 2000 x g for 10 minutes and assay. Dilute samples 1:20 000 with MIX Diluent as follows: Add 5 µl of sample to 495 µl of MIX Diluent (1:100) to make Solution A; then add 5 µl of Solution A to 995 µl of MIX Diluent (1:200) to make a final working solution (1:20 000). The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants; make an appropriate dilution and assay. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with 0.1 M phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract into MIX Diluent and assay. Freeze the remaining extract at -20^{0}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. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 320 ng of human Plasminogen Standard with 4 ml of MIX Diluent to produce 80 ng/ml of solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (80 ng/ml) twofold with equal volume of MIX Diluent to produce 40, 20, 10, 5, 2.5 and 1.25 ng/ml of solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[Plasminogen] (ng/ml)
P1	1 part Standard (80 ng/ml)	80.00
P2	1 part P1 + 1 part MIX Diluent	40.00
P3	1 part P2 + 1 part MIX Diluent	20.00
P4	1 part P3 + 1 part MIX Diluent	10.00
P5	1 part P4 + 1 part MIX Diluent	5.00
P6	1 part P5 + 1 part MIX Diluent	2.50
P7	1 part P6 + 1 part MIX Diluent	1.25
P8	MIX Diluent	0.00

- **Biotinylated Plasminogen Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^0C.
- **Wash Buffer Concentrate (20x):** 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 MIX Diluent. Any remaining solution should be frozen at -20^0C.

Assay Procedure

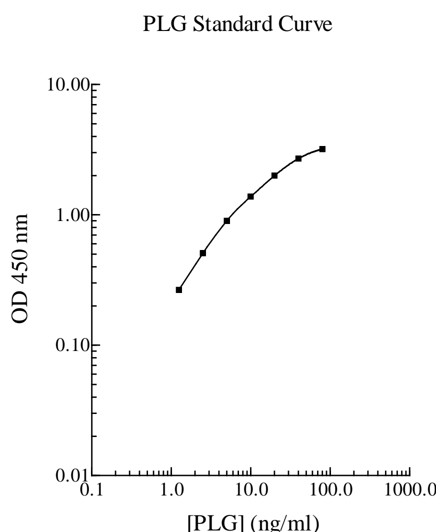
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature ($20\text{-}30^0$C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for one hour. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated Plasminogen Antibody to each well and incubate for one hour.
- Wash five times with 200 µl of Wash Buffer as 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 five times with 200 µl of Wash Buffer as above.
- 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.
- 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. Please note that some unstable black particles may be generated at high concentration points to reduce the readings after stopping the reaction for about 10 minutes.

Data Analysis

- Calculate the mean value of the 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 plasminogen is typically < 1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0 % and 6.5% respectively.
- This assay recognizes both natural and recombinant human plasminogen.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Plasma**
1:10000	102%
1:20000	102%
1:40000	100%

	Average Percentage of Expected Value
Sample Dilution	**Cell Culture**
1:10	102%
1:20	100%
1:40	101%

Recovery

Standard Added Value	2.5 - 25 ng/ml
Recovery %	82-116 %
Average Recovery %	99 %

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 7.0