AssayMax Human Plasminogen ELISA Kit

Catalog Number EP1200-1
Lot #

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 and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures plasminogen in 2.5 hours. A murine 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 murine 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 (200 ng, lyophilized).
- **Biotinylated Plasminogen Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human plasminogen (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate)**: A 100-fold concentrate (120 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard 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.

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Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 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 2,000x g for 10 minutes and assay. Dilute samples 1:10,000 with EIA Diluent as follows: Add 5 µl of sample to 495 µl of EIA Diluent (1:100) to make Solution A; then add 5 µl of Solution A to 495 µl of EIA Diluent (1:100) to make a final working solution (1:10,000). The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2,000x 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 14,000x g for 20 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract into EIA Diluent and assay. Freeze the remaining extract at -20^{0}C or below for up to 3 months.

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.

- **Standard Curve:** Reconstitute the 200 ng of human Plasminogen Standard with 1.25 ml of EIA Diluent to produce a 160 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 (160 ng/ml) twofold with equal volume of EIA Diluent to produce 80, 40, 20, 10, 5, and 2.5 ng/ml of solutions. EIA Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[Plasminogen] (ng/ml)
P1	1 part Standard (160 ng/ml)	160.00
P2	1 part P1 + 1 part EIA Diluent	80.00
P3	1 part P2 + 1 part EIA Diluent	40.00
P4	1 part P3 + 1 part EIA Diluent	20.00
P5	1 part P4 + 1 part EIA Diluent	10.00
P6	1 part P5 + 1 part EIA Diluent	5.00
P7	1 part P6 + 1 part EIA Diluent	2.50
P8	EIA Diluent	0.00

- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water.
- **Biotinylated Plasminogen Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 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.

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^{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 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 30 minutes.
- 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 approximately 7 to10 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.

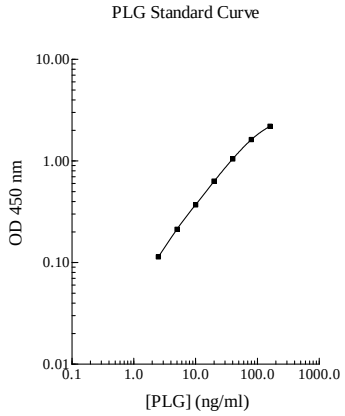
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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 4-parameter 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.



Sensitivity and Specificity

- The minimum detectable dose of plasminogen is typically < 1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0 % and 6.1% respectively.
- This assay recognizes both natural and recombinant human plasminogen. No significant cross-reactivity or interference was observed.

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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