Product Manual

Human Plasminogen ELISA Kit

Catalog Number

STA-393 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Plasminogen is a plasma glycoprotein 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. The plasminogen system plays a role in macrophage recruitment, arterial stenosis, atherosclerosis, aneurysm formation, skin and corneal wound healing, glomerulonephritis, and neovascularization.

Cell Biolabs' Human Plasminogen ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human plasminogen in plasma, serum or other biological fluid samples. The kit has a detection sensitivity limit of 150 pg/mL human plasminogen. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-362: Human ApoAI ELISA Kit
2. STA-363: Human ApoAII ELISA Kit
3. STA-364: Human ApoCI ELISA Kit
4. STA-365: Human ApoCII ELISA Kit
5. STA-366: Human ApoCIII ELISA Kit
6. STA-367: Human ApoE ELISA Kit
7. STA-368: Human ApoB-100 ELISA Kit
8. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
9. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
10. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human Plasminogen Antibody Coated Plate (Part No. 239301): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Human Plasminogen Antibody (1000X) (Part No. 239302): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.

7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human Plasminogen Standard (Part No. 239303): One 100 µL vial of 1 µg/mL Human Plasminogen in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum or Other Biological Fluids
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human Plasminogen Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Human Plasminogen Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Human Plasminogen antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of human Plasminogen standards in the concentration range of 0 to 10,000 pg/mL in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Human Plasminogen Standard (µL)	Assay Diluent (µL)	Human Plasminogen (pg/mL)
1	10	990	10,000
2	500 of Tube #1	500	5,000
3	500 of Tube #2	500	2,500
4	500 of Tube #3	500	1,250
5	500 of Tube #4	500	625
6	500 of Tube #5	500	313
7	500 of Tube #6	500	156
8	0	500	0

Table 1. Preparation of Human Plasminogen Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC up to three months. Normal plasma samples require about 20,000 to 40,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC up to three months. Normal serum samples require about 20,000 to 40,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC up to three months.

Assay Protocol

1. Prepare dilutions of plasma, serum or other biological fluid samples in PBS containing 0.1% BSA.
2. Add 100 µL of human Plasminogen unknown sample or standard to the Anti-Human Plasminogen Antibody Coated Plate. Each Plasminogen unknown sample, standard and blank should be assayed in duplicate.
3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-Human Plasminogen antibody to each well. Incubate at room temperature for 2 hour on an orbital shaker.
6. Wash the strip wells 3 times according to step 4 above.
7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
9. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

10. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human Plasminogen ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

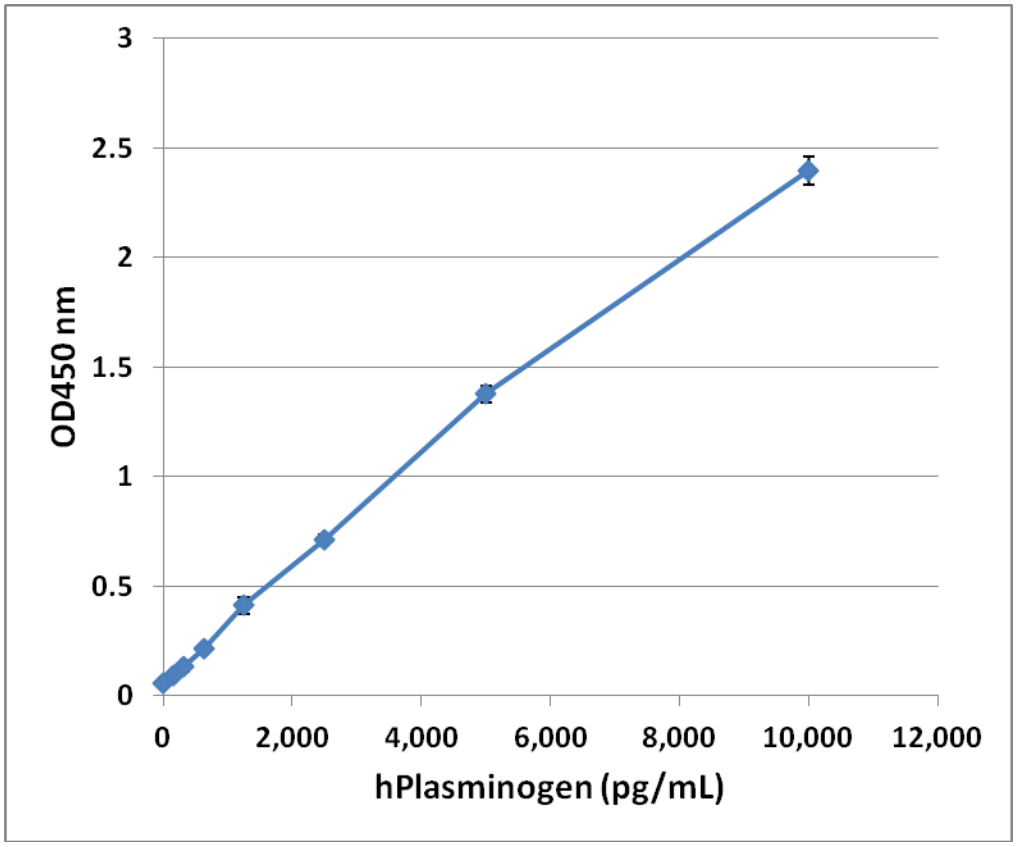

Figure 1: Human Plasminogen ELISA Standard Curve.

References

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