Human Plasminogen (PLG) ELISA Kit

Cat.No: DEIA9387V2
Lot. No. (See product label)

Size

96T

Intended use

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 Test

The Human Plasminogen ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human plasminogen in plasma, serum, urine, saliva, milk, CSF, cell culture, 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 washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Human Plasminogen Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human plasminogen.
2. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
3. Human Plasminogen Standard: Human plasminogen in a buffered protein base (128 ng, lyophilized).
4. Biotinylated Human Plasminogen Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human plasminogen (120 µl).
5. MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml).
6. Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
7. Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 µl).
8. Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
9. Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Materials Required But Not Supplied

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1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
3. Deionized or distilled reagent grade water.

Storage

1. Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
2. Store SP Conjugate and Biotinylated Antibody at -20°C.
3. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
4. 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.
5. Diluent (1x) may be stored for up to 30 days at 2-8°C.
6. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Specimen Collection And 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 MIX Diluent and assay. 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, and remove serum. Dilute samples 1:20000 with MIX Diluent and assay. 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 MIX Diluent and assay. Store samples at -20°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 minutes. Collect the supernatant and measure the protein concentration. The user should determine the optimal dilution factor. Dilute the tissue extract into MIX Diluent and assay. Freeze the remaining extract 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 and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Milk dilution is suggested at 1:40 in MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:40 into MIX Diluent and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Sample Dilution Guidelines below for further instruction.
Guidelines for Dilutions of 1:100 or Greater (for reference only; please follow the insert for specific dilution suggested)
1:100
A) 4 ul sample: 396 µl buffer(100x) = 100 fold dilution
Assuming the needed volume is less than or equal to 400 µl.

1:1000
A) 4 µl sample : 396 µl buffer (100x)
B) 24 µl of A : 216 µl buffer (10x) = 1000 fold dilution

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Assuming the needed volume is less than or equal to 240 µl.

1:10000
A) 4 µl sample : 396 µl buffer (100x)
B) 4 µl of A : 396 µl buffer (100x) = 10000 fold dilution
Assuming the needed volume is less than or equal to 400 µl.

1:100000
A) 4 µl sample : 396 µl buffer (100x)
B) 4 µl of A : 396 µl buffer (100x)
C) 24 µl of B : 216 µl buffer (10x) = 100000 fold dilution
Assuming the needed volume is less than or equal to 240 µl.

Reagent Preparation

1. Freshly dilute all reagents and bring all reagents to room temperature before use.
2. MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
3. Standard Curve: Reconstitute the 128 ng of Human Plasminogen Standard with 1.6 ml of MIX Diluent to produce an 80 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 (80 ng/ml) 1:2 with equal volume of MIX Diluent to produce 40, 20, 10, 5, 2.5, and 1.25 ng/ml solutions. MIX 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	[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	5.000
P6	1 part P5 + 1 part MIX Diluent	2.500
P7	1 part P6 + 1 part MIX Diluent	1.250
P8	MIX Diluent	0.000

4. Biotinylated Human Plasminogen Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
5. 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.
6. 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°C.

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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 Human Plasminogen Standard or sample per well. Cover wells with a sealing tape and incubate for 1 hour. Start the timer after the last 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 Human 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 15 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.

Typical Standard Curve

- 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 (OD) 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.

Typical Data
- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.
The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

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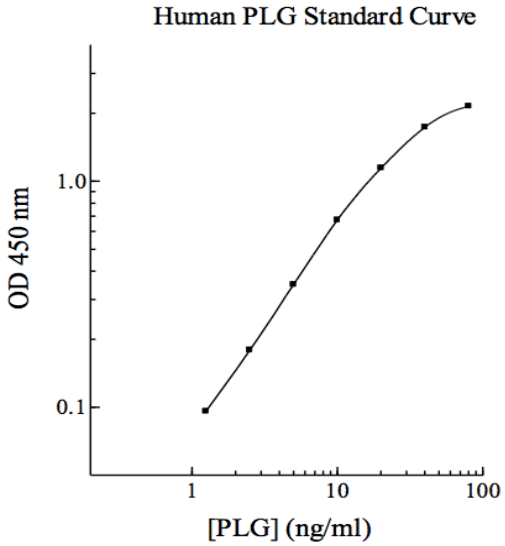
Standard Point	ng/ml	OD	Average OD
P1	80.00	2.168 2.118	2.143
P2	40.00	1.767 1.697	1.732
P3	20.00	1.141 1.144	1.143
P4	10.00	0.683 0.664	0.674
P5	5.000	0.350 0.348	0.349
P6	2.500	0.180 0.177	0.179
P7	1.250	0.095 0.096	0.096
P8	0.000	0.018 0.019	0.018
Sample: Pool Normal, Sodium Citrate Plasma (20000x)		0.537 0.569	0.553



Reference Values

- Normal human plasminogen plasma levels range from 60 to 215 μg/ml.
- Human plasma and serum samples from healthy adults were tested (n=40). On average, plasminogen level was 151 μg/ml.

Sample	n	Average Value (μg/ml)
Human Pool Normal Plasma	10	142
Human Normal Plasma	20	150
Human Pool Normal Serum	10	162

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Performance Characteristics

- The minimum detectable dose of plasminogen as calculated by 2SD from the mean of a zero standard was established to be 0.4 ng/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.2%	3.5%	3.6%	7.6%	7.9%	8.2%
Average CV (%)	3.4%			7.9%		

Specificity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	5%
Rat	None
Human	100%
Swine	None
Rabbit	None
Mouse	None
Proteins	**Cross Reactivity (%)**
Plasmin	<30%

Linearity

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1:10000	89%	96%
1:20000	99%	98%
1:40000	104%	106%

Recovery

Recovery was determined by spiking two plasma samples with different plasminogen concentrations.

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Sample	Unspiked Sample (ng/ml)	Spike (ng/ml)	Expected	Observed	Recovery (%)
1	5.0	2.5	7.5	7.8	104%
		10.0	15.0	15.7	105%
		30.0	35.0	33.4	95%
2	10.0	2.5	12.5	13.7	110%
		10.0	20.0	19.1	96%
		30.0	40.0	38.7	97%
Average Recovery (%)					101%

Precautions

1. This product is for Research Use Only and is Not For Use In Diagnostic Procedures.
2. Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
3. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
4. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
5. The Stop Solution is an acidic solution.
6. The kit should not be used beyond the expiration date.

References

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

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