TECHNOZYM® Glu-Plasminogen ELISA Kit

For research use only REF TC12040

3050087-014

Plasminogen is the inactive precursor of plasmin, the central enzyme responsible for fibrinolysis. Plasminogen, which is synthesized by the liver, is a 92,000 dalton single-chain glycoprotein. It circulates in plasma at a concentration of approximately 200 µg per mL with a half life of 2.2 days. Through the action of certain proteases such as tissue-type plasminogen activator or urokinase, a single peptide bond in the plasminogen molecule is cleaved to yield the two chain molecule, plasmin. Plasmin's major function is physiologic clot dissolution through the degradation of the insoluble polymer fibrin into soluble fragments. Thus, the concentration of plasminogen is one of the numerous factors critically influencing the rate of fibrinolysis in vivo.
Plasminogen levels are often depressed in both acute and chronic hepatic disease. This may be due either to decreased synthesis or increased consumption during disseminated intravascular coagulation. In primary biliary cirrhosis or early common bile duct obstruction plasminogen values are normal or even elevated. Abnormally low plasminogen values have been found in patients with generalized hyperfibrinolysis and neonates with Lipström syndrome.

Application

The Glu-plasminogen ELISA is useful for the determination of Glu-plasminogen levels, particularly in patients with thromboembolic complications, those undergoing lytic therapy or in cases of hyperfibrinolysis.

Test principle

The TC Glu-plasminogen ELISA test is a solid phase enzyme immunoassay.

Specificity

The monoclonal antibodies employed in this test system recognize only uncleaved Glu-plasminogen. Thus, the results are not affected by the presence of plasmin-alpha-2-antiplasmin complexes or plasmin-modified Lys-plasminogen. The assay measures Glu-plasminogen in a range from 0.06-0.5 µg/mL. Normal plasma levels are 60-250 µg/mL. The inter- and intra-assay variations are less than 10% and 5%, respectively.

Test samples

Use fresh EDTA plasma samples. For this purpose collect the blood of patients to be tested in precooled plastic or siliconilized tubes containing EDTA as anticoagulant in 1:10 ratio to blood. Centrifuge the blood within 90min. after the puncture at 2000g for 30min. at 4°C. Pipette off the plasma. Store aliquots at a temperature below -30°C. Thawing and refreezing of plasma aliquots is not recommended.
In this test plasma samples are used at 1:500 or 1:1000 dilution.
Haemolytic and lipaemic plasmas may be used. Do not use samples which contain clots or show any signs of coagulation. Since in certain conditions such as endogenous hyperfibrinolysis or during thrombolytic therapy, Glu-plasminogen may be degraded by proteinases after blood-drawing, it is advisable to add proteinase inhibitors (2000 units Aprotinin; 20mM benzamidine/mL blood) to samples.

Kit components

Determinations: 42 samples in duplicate

1. **PLATE + PLATE COVER**
   12 x 8 well microtitre strips precoated with a monoclonal anti-Plasminogen antibody and blocked with 1% bovine serum albumin (BSA), lyophilised. (TC-Code GX)
2. **STANDARD**
   1x lyophilized Normal Plasma, (TC-Code BJ)
3. **POX-ANTIBODY**
   1x conjugated polyclonal anti Plasminogen antibodies (concentrated). (TC-Code BG)
4. **INCUBATION BUFFER**
   (PBS; pH 7,3); contains stabiliser protein; 0.05% proclin; and blue dye, 1 bottle, 90ml, ready for use (TC-Code NB)
5. **SUBSTRATE - (green cap)**
   1x 12 mL TMB (Tetramethylbenzidine) in substrate buffer containing H_2O_2. Ready to use. (TC-Code KN)

6 **STOP SOLUTION -** (red cap)
1x 12 mL 0.45 mol/l Sulphuric Acid (TC-Code KK)

7. **WASHING BUFFER Concentrate-** (1+11,5)
   (PBS; pH 7,3) containing detergent; 0,01% merthiolat, 1 bottle, 80 ml (TC-Code NA)

Kit storage: Store all components at 2... 8° C.

Also required

1. Micropipettes and a multichannel micropipette; pipette tips.
2. Glass or plastic test tubes for diluting the standard+ samples.
3. Laboratory bottles or beakers and graduated cylinders for diluting wash and incubation buffer.
4. Distilled or deionised water.
5. Absorbent paper towels.
6. Microtitre plate washer (alternatively, washing can be performed manually using a multichannel pipette or repeating syringe).
7. A microtitre plate reader equipped with a 450nm filter and, if possible, a 620 nm reference filter.
8. A 37°C incubator
9. Graph paper.

Reagent handling & bench stability

All reagents must be at ambient temperature before use.

Material/ Reagent	State	Storage	Stability
Standard	after reconstitution	-20 °C	6 months
POX AB	working solution	room temperature	60 minutes
	after opening	2…8°C	6 months
ELISA test strip	after opening	2…..8 °C with adhesive film in plastic bag	expiry date
Washing buffer concentrate	after opening	2 ...8°C	6 months
Washing buffer	1+11,5 dilution of concentrate	2 ... 8 °C	3 weeks
Incubation buffer	after opening	2 ... 8 °C	2 months
Chromogenic Substrate	after opening	2 ... 8 °C	expiry date

RT = Room Temperature
POX AB = Peroxidase conjugated Antibody

STANDARD CURVE

Restore a vial of standard with exactly 1 mL distilled water.

Tube		Incubation buffer	Conc. µg/mL
Predilution	0.01 mL Standard	0.19 mL	5,5
A	0.055 mL Predilution	0.55 mL	0.5
B	0.3 mL from A	0.3 mL	0.25
C	0.3 mL from B	0.3 mL	0.125
D	0.3 mL from C	0.3 mL	0.063
E		0.3 mL	0

SAMPLE DILUTIONS

Dilute the plasma sample 1:500 or 1:1000 with incubation buffer

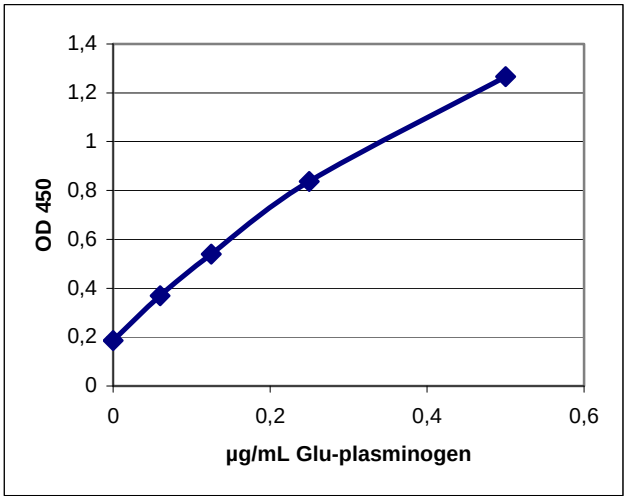
POX-CONJUGATED ANTIBODY

Preparing the conjugate working solution (1+50): Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer.

For 8 test wells: Mix 20 µL conjugate with 1000 µL incubation buffer

Assay Procedure:

1. **SAMPLE/ STANDARD ADDITION**
   Pipette 0.1 mL of the diluted samples/standard into separate wells. Running standard/sample in duplicate is recommended.
2. **SAMPLE INCUBATION**
   Cover the plate with a plastic foil and incubate overnight at 4°C.
3. **WASH PLATE**
   Reconstitute required strips by adding 0.25 mL of wash buffer to the wells and tip out the contents. Wash the strips four times further with wash buffer. Tap strips on absorbant paper and make sure the wells are completely dry.
4. **POX ANTIBODY ADDITION**
   Add 0.1 mL of the diluted POX anti-Plasminogen antibody to all wells, preferably with a multichannel pipette.
5. **POX ANTIBODY INCUBATION**
   Cover and incubate the plate for 1 hour at 37°C.
6. **WASH PLATE**
   Wash five times as described in step 3.
7. **SUBSTRATE**
   Pipette 0.1mL of TMB substrate to all wells. Incubate for 15 minutes at room temperature .
8. **STOP**
   Pipette 0.1 mL of stop solution to all wells.
9. **READ**
   Measure absorbances at 450nm (with 620nm reference filter if available). Read absorbances within one hour after the addition of the stop solution.
10. **GRAPH**
    Construct a graph of standard curve.
11. Locate the absorbance for each sample on the curve and read the corresponding value from the horizontal axis. Do not forget to multiply by the dilution factor (500) or (1000) for the samples.



Evaluation of results:

Normal Glu-plasminogen values range from 60-250 µg/mL plasma.

Time table **Summary of procedure**		***time required***	***Temp.***
Reagent, standard sample handling		1-2 hours	
1. Sample - incubation	100 µL	overnight*	4°C
wash 5 times	250 µL		
2. POX-AB – incubation	100 µL	1 hour	37°C
wash 5 times	250 µL		
3. Substrate – incubation	100 µL	15 minutes	RT
Stop solution	100 µL		
Read absorbances			
Time total: min.		**3hr+15min to-4 hours+15 min (+ 1 night)**	

Notes

Be sure to prepare all reagents before proceeding with the assay. It is critical to keep the time necessary for pipetting standards and samples to a minimum and avoid delays.
Be sure to wash the plate thoroughly and completely remove any residual wash buffer after each wash cycle. Insufficient washing can lead to erroneously high values and incomplete removal of wash buffer to irregularities due to the dilution of added reagents.
As mentioned use a multichannel pipette to add peroxidase conjugate, TMB substrate and stop solution

Warning

Potentially biohazardous material. Donor plasma used in this kit was tested by internationally approved methods for the presence of antibodies to HIV and hepatitis B virus and found to be negative. However, all human blood products should be handled as potentially infectious material.

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