

IMUCLONE® Glu-Plasminogen ELISA

Product No. 640

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INTENDED USE

The IMUCLONE® Glu-Plasminogen ELISA is an enzyme-linked sandwich immunoassay specific for the determination of native human glu-plasminogen levels, particularly in patients with thromboembolic complications, those undergoing lytic therapy or in cases of hyperfibrinolysis. The assay is designed to be used with citrated or EDTA treated plasma samples.

The assay is for research use only and not intended for diagnostic or therapeutic procedures.

BACKGROUND

Glu-plasminogen is the inactive precursor of plasmin, the central enzyme responsible for fibrinolysis. It is a 92,000 Dalton single-chain glycoprotein synthesized and secreted by the liver, circulating in plasma at a concentration of approximately 200 µg/mL with a half-life of 2.2 days. Cleavage by various proteases, including tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), yields the two-chain plasmin molecule. The major function of plasmin is clot dissolution through the degradation of fibrin, an insoluble polymer, into soluble fragments. Therefore, the glu-plasminogen concentration is a critical factor governing the rate of fibrinolysis *in vivo*.

Low levels of plasminogen are often associated with both acute and chronic hepatic disease where decreased liver synthesis and increased consumption during disseminated intravascular coagulation (DIC) occur. Abnormally low plasminogen levels have been found in patients with hyperfibrinolysis and newborns with Lipström syndrome.

PRINCIPLE

The IMUCLONE Glu-Plasminogen ELISA kit employs a murine monoclonal antibody, coated to plastic microwells, specific for the N-terminal domain of glu-plasminogen, which is cleaved from the molecule upon activation and is not present in lys-plasminogen, plasmin or complexes. Samples incubate in the precoated microwells. Extraneous plasma proteins are washed away and a second, horseradish peroxidase (HRP) conjugated monoclonal antibody recognizing a different epitope of the glu-plasminogen molecule is added, completing the formation of the antibody sandwich complex.

The addition of a 2-2' azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) substrate, and its subsequent reaction with the HRP creates a green colored solution. Glu-plasminogen levels are quantified by measuring solution absorbances at 405 nm and comparing the values with those from a standard curve.

REAGENTS

- 12 x 8 well precoated microtest strips with frame
- 1 vial Glu-Plasminogen Standard (lyophilized), see labeled concentration
- 1 vial Detection Antibody, HRP conjugated anti-human plasminogen IgG
- 1 vial Substrate, ABTS (12 mL)
- 1 vial Stop Solution, 0.32% Sodium Fluoride (12 mL)
- 1 vial Wash Buffer, 0.15M PBS, 0.05% Tween 20, pH 7.2 (20 mL, 12.5x concentrate)
- 2 vials Dilution Buffer, 0.15M PBS, 1% BSA (20 mL, 2.5x concentrate)

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- 0.22 µm filtered deionized or distilled H₂O (di H₂O)
- 50-200µL eight channel multi-pipette
- 10-200µL single pipette
- Plastic test tubes
- Microwell plate reader at 405 nm

REAGENT PREPARATION

A. Wash Buffer

Add the 20 mL of concentrated buffer to 230 mL of filtered di H₂O.

B. Dilution Buffer

Add the 20 mL of concentrated buffer to 30 mL of filtered di H₂O.

C. Glu-Plasminogen Standard

1. Add 0.5 mL of filtered di H₂O to the vial of lyophilized plasminogen standard. Allow to stand for 15 minutes. Mix well.
2. Prepare serial dilutions of the standard in Dilution Buffer in labeled tubes as detailed in the table below. **IMPORTANT:** These instructions are for the lot specific standard which yields 11 µg/mL upon reconstitution.

Note: Use plastic or siliconized glass tubes for diluting the standards and plasma samples.

Tube	Glu-Plasminogen Standard	Volume of	Added to Volume of Dilution Buffer
A	0.500 µg/mL	0.09 mL of Standard	1.89 mL
B	0.250 µg/mL	0.30 mL of Tube A	0.30 mL
C	0.125 µg/mL	0.30 mL of Tube B	0.30 mL
D	0.063 µg/mL	0.30 mL of Tube C	0.30 mL
E	0.000 µg/mL	None	0.30 mL

WARNING: The glu-plasminogen provided in this kit is of human origin. Each donor unit used in the manufacture of this reagent has been tested by an internationally approved method and found to be negative for the presence of antibodies to Hepatitis B surface Antigen (HBsAg) and Human Immunodeficiency Virus (HIV). As no known method can offer complete assurance that products derived from human blood will not transmit HBsAg, HIV or other blood-borne pathogens, this plasma reagent should be handled as recommended for any potentially infectious human serum or blood specimen.

E. Detection Antibody

Add 1.2 mL of Dilution Buffer to the vial of Detection Antibody and mix well. This is now a 10x concentration of Detection Antibody. If you will not use all 12 microwell strips provided in the kit, aliquot the Detection Antibody in 100 µL amounts and freeze at -20°C. Before using, thaw the aliquots at 37°C.

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REAGENT STABILITY

Store unused microwell strips and unreconstituted reagents at +2° - +8°C until the expiration dates indicated on labels. Store reconstituted reagents at +2° - +8°C in the dark for up to one month, except for the Detection Antibody which should be aliquoted and frozen at below -20°C.

SAMPLE PREPARATION

Plasma Samples

Dilute plasma samples 1:400 to 1:800 with Dilution Buffer.

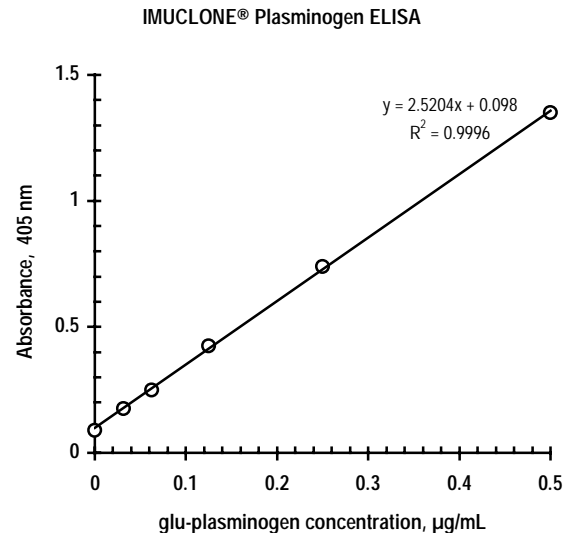
Note: Hemolytic and lipemic plasmas may be used in the assay. Do not use plasmas which contain clots or show signs of coagulation. Under certain conditions, eg. endogenous hyperfibrinolysis or thrombolytic therapy, glu-plasminogen may be degraded by proteases after blood-drawing. Adding a protease inhibitor such as Aprotinin (2000 units/mL) is advisable.

ASSAY PROCEDURE

1. Open the foil pouch and remove the frame with the microwell strips. Remove the strips which will not be used and replace in the foil pouch. Tightly reseal the foil pouch and store at +2° - +8°C.
2. Wash wells 4 times with Wash Buffer.
3. Add 100 µL of either diluted standard or diluted plasma sample into microwell wells, cover the strips with clear plastic foil and incubate for 1 hour at 37°C. Run standards and samples in duplicate.
4. Wash wells 4 times with wash buffer.
5. Dilute aliquots (or all) of Detection Antibody 1:10 with Dilution Buffer. Mix well. Add 100 µL of diluted Detection Antibody to each well, cover and incubate for 60 minutes at 37°C. Discard any unused diluted Detection Antibody.
6. Wash wells 4 times with wash buffer.
7. Add 100 µL of ABTS substrate solution to each well, cover and incubate for 15 minutes at room temperature.
8. Stop the enzymatic reaction by adding 100 µL of Stop Solution. Tap the sides of the wells to ensure even distribution of the Stop Solution. Read the absorbances on a microwell plate reader at a wavelength of 405 nm within 30 minutes. Deduct the background average of the blanks from the standards and sample readings.

REPRESENTATIVE STANDARD CURVE

The standard curve is constructed by plotting the mean absorbance value calculated for each plasminogen standard versus the corresponding plasminogen concentration. Interpolate the plasminogen concentration for the diluted sample directly from the standard curve. A standard curve should be generated each time the assay is run. The following curve is for demonstration purposes only.



EXPECTED VALUES

The glu-plasminogen concentration in fresh normal plasma ranges from 60-250 µg/mL.

PERFORMANCE CHARACTERISTICS

Precision

Studies evaluating the intra-assay and the inter-assay variations of this ELISA found the following:

Intra-Assay Variation	< 5%
Inter-Assay Variation	< 10%

SPECIFICITY

The monoclonal antibodies used in the ELISA only recognize uncleaved glu-plasminogen, Plasmin-alpha-2-antiplasmin complexes or plasmin modified lys-plasminogen are not measured by the ELISA and do not affect the results