INTENDED USE

The 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

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

Diluted samples are added to microwells coated with a monoclonal antibody against human Glu-Plasminogen. During an incubation period, Glu-Plasminogen present in the sample will bind to the antibody coated to the wells. Following a washing step, a streptavidin-horseradish peroxidase (HRP) conjugated polyclonal anti-Glu-Plasminogen is added to the microwells to complete the formation of the antibody sandwich complex.

Following another washing step, the addition of a perborate-3,3’-5,5’-tetramethylbenzidine (TMB) substrate and its subsequent reaction with the HRP present generates a blue colored solution. The reaction is stopped by adding citrate stop solution, which turns the solution color yellow. Measuring the solution absorbance at 450 nm and extrapolating the value with those of a standard curve determines the level of Glu-Plasminogen present in the sample.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

0.22 µm filtered deionized or distilled water
50-200 µL eight channel multi-pipette, 10-200 µL single pipette
plastic test tubes, laboratory beakers, graduated cylinders
37°C incubator
graph paper
microwell plate washer
microwell plate reader with a 450 nm, if possible a 620 nm reference filter

REAGENT PREPARATION AND STORAGE

Precoated Microwell Strips: Once removed from the foil pouch, the microwell strips must be used within 60 minutes. Unused strips may be stored at 2°-8°C until the expiration date when sealed in the original pouch with the desiccant present, protected from any moisture.

Wash Buffer: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume deionised or distilled water (1+11.5). Mix well. (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37 °C within 10 minutes. Once opened, the Wash Buffer concentrate may be used for up to 6 months when stored at 2°-8°C. Diluted Wash Buffer may be stored at 2°-8°C for up to 3 weeks.

Dilution Buffer: Supplied ready to use. Once opened, the Dilution Buffer may be used for up to 2 months when stored at 2°-8°C.

Glu-Plasminogen Standard: Add 1.0 mL of filtered deionised or distilled water to the vial of lyophilized plasminogen standard. Allow to stand for 15 minutes. Mix well. Standard may be aliquoted and stored at –20°C for up to six (6) months.

Prepare serial dilutions of the standard in labeled tubes as detailed in the table below. IMPORTANT: These instructions are for the lot specific standard which yields 110 µg/mL upon reconstitution.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Volume of Dilution Buffer</th>
<th>Concentration µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predilution</td>
<td>0.19 mL</td>
<td>5.5</td>
</tr>
<tr>
<td>A</td>
<td>0.055 mL of Predilution</td>
<td>0.5</td>
</tr>
<tr>
<td>B</td>
<td>0.3 mL of Tube A</td>
<td>0.25</td>
</tr>
<tr>
<td>C</td>
<td>0.3 mL of Tube B</td>
<td>0.125</td>
</tr>
<tr>
<td>D</td>
<td>0.3 mL of Tube C</td>
<td>0.063</td>
</tr>
<tr>
<td>E</td>
<td>None</td>
<td>0</td>
</tr>
</tbody>
</table>

WARNING: The Glu-Plasminogen Standards provided in this kit is of human origin. Donor plasma used in this kit was tested by internationally approved methods for the presence of antibodies to Hepatitis B virus and Human Immunodeficiency Virus (HIV) and found to be negative. 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.

Antibody Conjugate: Prepare sufficient working strength Antibody Conjugate by diluting the concentrate 1+50 with Dilution Buffer. For using only a single 8 well strip, dilute 20 µL of concentrated conjugate with 1000 µL of Dilution Buffer. For using all 96 wells, dilute 240 µL of concentrated conjugate into 12 mL of Dilution Buffer. Once opened, the Antibody Conjugate may be used for up to 6 months when stored at 2°-8°C. Working strength Antibody Conjugate is stable for up to 1 hour at 20°-25°C.

Substrate: Supplied ready to use. The Substrate is stable until the stated expiration date when stored at 2°-8°C.

Stop Solution: Supplied ready to use. The Stop Solution is stable until the stated expiration date when stored at 2°-8°C.
SPECIMEN COLLECTION AND PREPARATION

Use fresh EDTA plasma samples. For this purpose collect the blood of patients to be tested in precooled plastic or siliconized tubes containing EDTA as anticoagulant:

1. Collect 9 parts of blood into 1 part of EDTA.
2. Centrifuge the blood within 90 min after the puncture at 2,000 x g for 30 minutes at 4 °C.
3. Pipette off the plasma.
4. Plasma should be stored at 2° - 8°C and assayed within 2 hours. Alternatively, plasma may be stored at below -30°C for up to 6 months. Thawing and refreezing of plasma aliquots is not recommended.

Dilute plasma samples 1:500 to 1:100 with Dilution Buffer.

Note: Hemolytic and lipemic plasmas may be used in the assay. Do not use plasmas that contain clots or show signs of coagulation. Under certain conditions, e.g. 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) or Benzamidine (20 mM) is advisable.

ASSAY PROCEDURE

1. Open the foil pouch and remove the frame with the microwell strips. Remove the strips that will not be used and replace in the foil pouch. Tightly reseal the foil pouch and store at 2° - 8°C.
2. Add 100 µL of either diluted standard or diluted plasma sample into separate microwells, cover the wells with clear plastic foil and incubate for overnight (12-16 hours) at 4°C. Standards and samples should be assayed in duplicate.
3. Wash the microwells 5 times with Wash Buffer (250 µL per wash).
4. Add 100 µL of working strength Antibody Conjugate to each well, cover and incubate for 1 hour at 37°C. Discard any unused working strength Antibody Conjugate.
5. Wash the microwells 5 times with Wash Buffer (250 µL per wash).
6. Add 100 µL of TMB Substrate to each well, cover and incubate for 10 minutes at 20°-25°C.
7. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well. Tap the sides of the wells to ensure even distribution of the Stop Solution.
8. Read the absorbances on a microwell plate reader at a wavelength of 450 nm (with 620nm reference filter if available) within 1 hour.

RESULTS

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.

CALCULATIONS

Interpolate the glu-plasminogen concentration of the diluted plasma sample directly from the standard curve. A curve regression software function that is included with the microwell plate reader may be used to calculate the concentrations. Multiply this calculation by the dilution factor (500 or 1000) to obtain the glu-plasminogen concentration of the neat plasma sample.

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 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 only 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.

Assay range

The assay measures Glu-plasminogen in a range from 0.06 - 0.5 µg/mL.