DIAGON
Solutions beyond imagination

INSTRUCTION FOR USE

Dia-PLASMINOGEN

Chromogenic reagent

CE

Cat. No.: 07508 Dia-PLASMINOGEN 4x2 ml

Intended Use

The Dia-Plasminogen kit is intended for the quantitative determination of plasminogen in human plasma using a chromogenic assay method.

Summary

Plasminogen (PLG) is the plasma precursor for the fibrinolytic enzyme, plasmin. The presence of an excess amount of streptokinase creates a complex which catalyses the reaction with the substrate to release p-nitroaniline (pNA). Plasminogen levels may be reduced due to congenital or acquired deficiency such as primary or secondary fibrinolysis. Plasminogen depletion also occurs during thrombolytic therapy, but the most common cause is disseminated intravascular coagulation (DIC).

Principle

The plasminogen-streptokinase complex catalyses the release of pNA from the chromogenic substrate.[1, 2] This rate of activity can be measured photometrically at 405 nm, and is directly proportional to the amount of plasminogen present in the sample. The activity can be monitored as an initial rate method or as an acid stopped method. The enzymatic activity is not sensitive to plasma inhibitors.[2, 3]

Warnings

The reagents contained in this kit are for *in vitro* diagnostic use only under professional's leadership.

Precautions

Potentially dangerous material – **DO NOT INGEST!**
Plasma products have been screened and found negative for the presence of Hepatitis B Antigen (HbsAg) HIV 1 and 2 antibody and HCV antibody; however they should be handled with the same precautions as a human plasma sample. During the use of deficient plasmas please keep the precautions for infectious materials treatment.

Waste Material Treatment

Refer to the product safety data sheets for risk and safety phrases and disposal.

Material Provided

Dia-PLG Streptokinase (4 x 2mL)
Dia-PLG Substrate (4 x 2mL)
Dia-PLG Buffer (4 x 5mL)

Dia-PLG Streptokinase

Ingredients: Each vial contains 10,000 IU lyophilized streptokinase with <1% human albumin as a stabiliser.
Preparation for Use: Reconstitute each vial with 2.0 mL deionised water.

Storage and Stability

The Streptokinase Reagent is stable until the expiration date printed on the vial label when stored at 2-8°C. The reconstituted reagent is stable for one week at 2-8°C or one month at -20°C.

Dia-PLG Plasmin Substrate
Ingredients: Each vial contains 5 µmol lyophilised H-D-Nle-HHT-Lys-pNA.2AcOH
Preparation for Use: Reconstitute each vial with 2.0 mL deionised water.

Storage and Stability
The substrate is stable until the expiration date printed on the vial label when stored at 2-8°C. The reconstituted reagent is stable for one week at 15-30°C, or two months at 2-8°C. If frozen immediately after reconstitution, the reagent is stable for 6 months and -20°C.

Dia-PLG Plasmin Buffer
Ingredients: The buffer is a 10-fold concentrate. After dilution, the buffer contains 0.05 M Tris-HCl, 0.110 M NaCl
Preparation for Use: Dilute each vial of the concentrated buffer to 50 mL with deionised water. After dilution, the buffer contains 0.05 M Tris-HCl, 0.110 M NaCl.

Storage and Stability
Diluted buffer is stable for one week at 18-25°C or one month at 2-8°C.

Specimen collection and handling [4]
Specimen: Plasma obtained from whole blood collected with 3.2% sodium citrate as an anticoagulant is the specimen of choice.
Specimen Collection: Blood may be collected with evacuated test tubes, a 2-syringe technique, or with a butterfly and syringe technique.

Accurate coagulation studies depend on the correct whole blood to anticoagulant ratio. For blood specimens with hematocrits (HCT) of 40-50% (normal), 9 parts of freshly collected whole blood should be immediately added to one part anticoagulant.

For blood specimens with hematocrits outside the normal range, adjust the amount of whole blood added to the anticoagulant according to the following formula:[5]

$$\text{Parts whole blood to one part anticoagulant} = \frac{0.6}{(1-.\text{HCT})} \times 9$$

If the hematocrit is abnormal, blood should be drawn into a syringe and an appropriate amount mixed with an adjusted volume of citrate anticoagulant.

Specimen Preparation: Centrifuge the whole blood specimen at an appropriate rcf and length of time to obtain platelet poor plasma (i.e. 3000 X g for 10 minutes). Immediately separate the plasma from the blood cells, and place plasma in a plastic test tube with cap.

Storage and Stability: Perform the assay within 2 hours. The plasma sample should be stored in capped plastic test tubes at 2 to 8°C if not tested immediately.
If testing is delayed for more than 2 hours, plasma may be stored at -20°C for up to one month. Thaw quickly at 37°C prior to testing, but do not allow to stand at 37°C for more than 5 minutes.

Limitations: Erroneous results may be caused by contamination with tissue fluids or stasis. Icteric, hemolytic or lipaemic specimens may interfere with test results. Avoid agitation, air bubbles or foaming. For the effects of commonly administered drugs, refer to Young, et al.[6]

Materials required but not provided:

Dia-CAL Spec (Cat. No.: 09605) or Normal Human Plasma assayed against a WHO Traceable Standard or NIBSC Standard

Control Plasmas:

Cat. No.: 09305: Dia-CONT Spec N
Cat. No.: 09405: Dia-CONT Spec P

Procedure

Assay calibration

Pooled Normal Plasma (PNP) that has been collected in the same way as plasma to be tested may be used for preparation of the plasminogen standards. Commercially prepared plasma standards in which the plasminogen level has been determined may also be used.

Procedure – Semi-Micro Cuvette End-Point Method

Allow reagents and plasma to warm to room temperature immediately before use.

1. Add 200 µl of Reference Plasma or test plasma to a test tube and incubate at 37°C for 2-4 minutes.

2. Add 200 µL of Streptokinase Reagent. Mix and incubate at 37°C for 2 minutes.

3. Add 200 µL of Plasmin Substrate. Mix gently and incubate at 37°C for 1 minute.

4. Add 200 µL of 50% acetic acid to stop the reaction.

5. (Optional) Add 200µL of water, as some spectrophotometers require a minimum of 1 mL in the cuvette.

6. Read absorbance at 405nm in a 1cm semi-micro cuvette. Use purified water to blank the spectrophotometer.

Results

Reference Plasma	PNP	Plasmin Buffer
100%	100 µl	500 µl
50%	200 µl of 100% Standard	200 µl
0%	--------	5000 µl

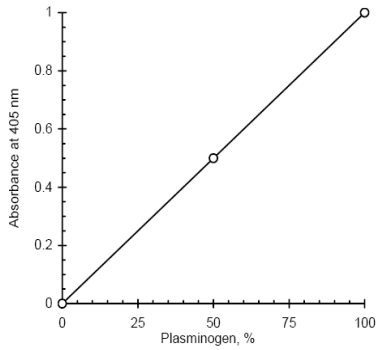
Test Plasma Concentration	Test Plasma	Plasmin Buffer
100%	100 µl	500 µl

Representative Standard Curve

Plot the absorbance obtained for each Reference Plasma against the percent of plasminogen on linear graph paper. Interpolate the plasminogen level of the unknown test plasma sample from the calibration curve. If a commercial plasminogen reference standard was used, adjust the plasminogen value determined for the test plasmas as follows:

% PLG (adjusted) =
% PLG (test plasma) x % PLG (reference)/100

The calibration curve shown below is for example only. A new calibration curve must be constructed each time the assay is performed.



Performance characteristics

Sensitivity
Dia-Plasminogen is manufactured to obtain a linear standard curve for plasminogen levels between 0 and 100%.

Specificity
Specificity is ensured by the use of a chromogenic substrate that is known to be specific for plasmin.

References
1. Soria, J.,Soria, C. and Samama, M., Pathologie Biologie 24:725, 1976.
2. Teger-Nilsson, A.C., Friberger, P. and Gyzander, E., Scand J ClinLabInvest 37:402, 1977.
3. Steinbuch, M., Friess, A., Druet, J. and Amouch, P., In "Proc. of the Vth Congress on Thrombosis and Haemostasis", Paris, Abst. 455, 1975.
4. NCCLS, Collection, Transport and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, H21-A2.
5. Triplett, D.A. ED., Standardization of Coagulation Assays: An Overview, College of Am Path, Skokie, IL. pg. 4, 1982.
6. Young, D.S. et al., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., AACC Press, Washington, D.C., 1990.

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Symbols			
IVD	In vitro diagnostics devices		Check in user manual
	Biohazard		Temperature range
	Manufacturer		Expiry date
LOT	LOT number	CE	CE conformity sign
REF	Catalogue number		