

Intended use of the kit

For the quantitative determination of plasminogen (Plg) activity in human citrated plasma using microplate, test tube and automated methods.

Background and summary

Measurement of plasminogen levels may aid in the diagnosis of fibrinolytic (blood clotting) disorders. Plasminogen is the precursor protein which after activation by plasminogen activators such as t-PA, u-PA or streptokinase, will form plasmin. The serine protease plasmin is a potent enzyme with a wide range of physiological functions. It is important to measure plasminogen levels since abnormal plasminogen activity is associated with different clinical circumstances.

Measurement principle

The plasminogen present in the sample is activated by the addition of an excess of streptokinase (Sk) forming a plasminogen-streptokinase (Plg/Sk) complex. Plasminogen-depleted fibrinogen is included in the streptokinase reagent in order to avoid the risk of overestimation of plasminogen in the pathological plasmas containing elevated levels of fibrinogen (Fib) and/or fibrin degradation products (FDP). The Plg-Sk/Fib complex is determined by the rate of hydrolysis of the chromogenic substrate S-2403.

1. Plg + Sk/Fib (excess) → [Plg-Sk/Fib]
2. S-2403 $\xrightarrow{[Plg-Sk/Fib]}$ peptide + pNA

The pNA release measured at 405 nm is proportional to the plasminogen level of the plasma sample.

Reagents

1. **S-2403 8.4 mg** 2 vials
Chromogenic substrate pyroGlu-Phe-Lys-pNA HCl lyophilized with mannitol as bulking agent.
2. **Streptokinase 20 000 IU/Fibrinogen 3 mg** 2 vials
Contains human albumin, Tween 80 and a fibrin polymerization inhibitor.

Reagent preparation

For microplate and test tube techniques reconstitute with 5.0 mL of water (see REAGENTS 3). Replace the stoppers and swirl gently. Make sure of the complete reconstitution of the product. Keep the reagent at 15-25°C for 10-30 min and invert before use.

NOTE: Other reagent reconstitution volumes may apply for automated methods. The reagents are not interchangeable between lots.

PRECAUTIONS AND WARNINGS

Each donor unit used in the preparation of human source reagent has been tested by FDA approved methods for the presence of Hepatitis B surface antigen and antibodies to HIV 1 and 2 and Hepatitis C and found to be negative. However, since no test can completely rule out the presence of these blood born diseases, the handling and disposal of human source reagents from this product should be made with care. Harmful if swallowed (R 22). Avoid contact with skin and eyes (S24/25). Do not empty into drains (S29). Wear suitable protective clothing (S36). This product is for *in vitro* diagnostic use.

Reagents required but not provided:

3. Deionized water, filtered through 0.22 µm or NCCLS type II water⁵
4. Saline (0.9% NaCl)
5. Calibrated human normal plasma
6. Appropriate controls calibrated for plasminogen activity
7. Acetic acid 20% or citric acid 2% (end-point method)

Materials required but not provided:

- Spectrophotometer 405 nm (and 490 nm for microplate procedure)
- Incubator 37°C ± 0.2°C
- Microplates⁵ or semi-micro cuvettes
- Centrifuge, 2000 x g
- Plastic test tubes
- Stopwatch
- Vortex mixer
- Calibrated pipettes
- Linear graph paper

*NOTE: Do not use microplates intended for coating!

Storage conditions and stability

The sealed reagents are stable at 2-8°C until the expiry date printed on the label. Avoid contamination by microorganisms in the reagents.

1. S-2403
Stability after reconstitution: 6 months at 2-8°C in the original vial.
2. Streptokinase/Fibrinogen
Stability after reconstitution: 1 month at 2-8°C in the original vial.

WARNING: Do not use reagents beyond the expiry date printed on the package label. Discard if the substrate solution appears yellow.

Specimen collection

Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Centrifugation: 2000 x g for 10-20 minutes at 20-25°C. Refer to NCCLS document H21-A2 for further instructions on specimen collection, handling and storage.

Quality control

Normal and abnormal controls are recommended for a complete quality control program. Assigned values of Controls should be traceable to the International Standard. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least every 8 hour shift in accordance with good laboratory practice. Refer to Westgard et al for identification and resolution for out of control situations.

Results

Plasminogen results are reported in activity (%).

Expected values

Normal range for plasminogen in plasma is 80-120%. For Coamatic Plasminogen a normal range study was performed using the Cobas Mira instrument:

n	%
50	70-138 (mean ±2SD)

Due to many variables which may affect results, each laboratory should establish its own normal range.

Procedure

All performance characteristics included in this package insert are referred to Cobas Mira. Detailed instrument settings including instructions for preparation of the reagents for a variety of automated instruments are available on request from Chromogenix

Calibration

A standard curve is obtained by analyzing different dilutions of calibrated normal plasma in saline. Before assay, the mixtures have to be diluted further by adding 50 µL to 2000 µL saline as described in the PROCEDURE section. A 132% plasminogen standard dilution is obtained by adding 50 µL normal plasma to 1500 µL saline.

NOTE: After reconstitution of the Sk/Fib reagent the standard curve obtained can be used for 4 weeks provided that controls (normal and abnormal) are analyzed in each run. If a control value deviates from the accepted range, a new standard curve has to be performed.

Dilution of samples and controls

Samples/controls/standards	50 µL
Saline	2000 µL
Mix well	

REMARK: Samples with plasminogen activity above 135% should be prediluted 1+1 in saline and the results recalculated accordingly.

Microplate method

Diluted samples/controls/standards	50 µL
Incubate at 37°C for 3-4 min	
Sk/Fib (pre-heat at 37°C)	50 µL
Incubate at 37°C for 180 sec	
S-2403 (pre-heat at 37°C)	50 µL
A. Kinetic method: read ΔA/min at 405 nm for 30-120 sec	
B. End-point method: proceed as described below	
Incubate at 37°C for 180 sec	
Acetic acid 20% or 2% citric acid	50 µL
Mix	

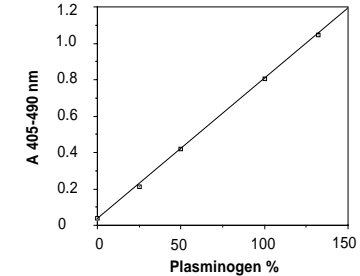
Read the absorbance against water at 405 nm. If possible, read and subtract the absorbance at 490 nm in order to compensate for differences in the material of the microplate wells.

Test tube method

Use 200 µL instead of 50 µL for all pipetting steps.

Calculation

Plot the change in absorbance per minute (ΔA/min) or absorbance (A) for the standard samples against their concentration of plasminogen on linear graph paper. Plot ΔA/min or A on the Y axis and % plasminogen on the X axis. Connect the standard points with the best fit straight line. Samples are evaluated based on this standard curve. An example of a typical standard curve (microplate method) is shown below.



Absorbance values for the standard curve should be within the following limits:

Standard	Microplate method Absorbance	Test tube method Absorbance
0%	< 0.10	< 0.10
100%	0.6-1.2	1.0-1.7

Performance Characteristics

LIMITATIONS/INTERFERING FACTORS

Plasminogen results are not affected by elevated levels of fibrinogen, heparin up to 2 U/mL, LMW heparin up to 2 U/mL, hemoglobin up to 200 mg/dL, bilirubin up to 20 mg/dL, FDP's up to 30 mg/mL and triglycerides up to 1000 mg/dL. The assay is not influenced by icteric plasmas. However, sample blank activities should be determined and subtracted when analyzing severe hemolytic or lipemic plasmas when the end-point method is used. The sample blank activity is determined following the method procedure but substituting the Sk/Fib reagent with the same volume of water (see REAGENTS 3.).

Precision

The precision of the method has been determined on two different analyzers, n=35.

Analyzer A	Within run CV (%)	Between run CV (%)	Total CV (%)
Mean concentration	1.9	1.0	2.1
49%	1.5	0.9	1.8
96%			
Analyzer B	Within run CV (%)	Between run CV (%)	Total CV (%)
Mean concentration	1.9	5.0	5.1
100%	1.6	4.1	4.1

These results have been obtained at Chromogenix laboratories and should be considered as examples only. Other laboratories are requested to establish their own precision data.

Correlation

The assay shows a good correlation with Coatest Plasminogen. The comparison is based on plasma samples (from healthy individuals and from patients with expected low levels of plasminogen):

Coamatic Plasminogen (ACL) % Plg (Coamatic) = -9.8 + 0.94x (Coatest)	r=0.92, n=59
Coamatic Plasminogen (Cobas Mira S) % Plg (Coamatic) = -7.5 + 1.05x (Coatest)	r=0.97, n=41
Coamatic Plasminogen (Cobas Bio) % Plg (Coamatic) = -4.7 + 1.02x (Coatest)	r=0.97, n=30
Coamatic Plasminogen (Cobas Fara) % Plg (Coamatic) = -4.4 + 1.14x (Coatest)	r=0.97, n=30
Coamatic Plasminogen (Epos Analyzer 5060*) % Plg (Coamatic) = -15.6 + 1.08x (Coatest)	r=0.99, n=32
Coamatic Plasminogen (MLA Electra 900C/1000C) % Plg (Coamatic) = -17.0 + 1.13x (Coatest)	r=0.98, n=37
Coamatic Plasminogen (Test tube) % Plg (Coamatic) = -18.0 + 1.15x (Coatest)	r=0.92, n=23
Coamatic Plasminogen (Microplate) % Plg (Coamatic) = -14.7 + 1.05x (Coatest)	r=0.93, n=31

*Not available in all countries

Recommended measuring range

The relationship between the pNA release, measured at 405 nm as change in absorbance (ΔA/min) or absorbance (A), and the plasminogen concentration is linear in the 0-135% range of normal plasma.

Detection limit

The assays allow detection of at least 5% plasminogen.

Sensitivity:

System

Cobas Mira ΔAbs per 1% Plasminogen activity: 0.01 ΔAbs

Determinations/kit

Microplate method: 200 Test tube method: 50

Bibliography / Literatur / Bibliografía / Bibliographie / Bibliografia / Bibliografia / Litteratur / Litteraturförteckning / Βιβλιογραφία

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Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / χρησιμοποιηθέντα σύμβολα

IVD	LOT				CONTROL			EC REP
<i>In vitro</i> diagnostic medical device <i>In-vitro</i> Diagnostikum De uso diagnóstico <i>in vitro</i> Dispositif mèdical de diagnostic <i>in vitro</i> Per uso diagnostico <i>in vitro</i> Dispositivo médico para utilização em diagnóstico <i>in vitro</i> "in vitro" diagnostisk udstyr <i>In vitro</i> diagnostisk medicinsk produkt Προϊόν για διαγνωστική χρήση <i>In vitro</i>	Batch code Chargen-Bezeichnung Identificación número de lote Désignation du lot Numero del lotto Número de lote Batch nr. Tillverkningskod Αρ. Παρτίδας	Use by Verwendbar bis Caducidad Utilizzabile jusqu'à Da utilizzare prima del Data límite de utilização Anvendelse Användning Χρήση έως	Temperature limitation Festgelegte Temperatur Temperatura de Almacenamiento Températures limites de conservation Limiti di temperatura Limite de temperatura Temperatur begrænsninger Temperatur gräns Περιορισμοί θερμοκρασίας	Consult instructions for use Beilage beachten Consultar la metódica Lire le mode d'emploi Vedere istruzioni per l'uso Consultar as instruções de utilização Se vejledning for anvendelse Ta del av instruktionerna före användning Συμβουλευτήτε τις οδηγίες χρήσης	Control Kontrollen Control Contrôle Controllo Controlo Kontrol Kontrol Υλικό ποιοτικού ελέγχου	Biological risks Biologisches Risiko Riesgo biológico Risque biologique Rischio biologico Risco biológico Miljø oplysninger Biologiska risker Βιολογικοί κίνδυνοι	Manufacturer Hergestellt von Fabricado por Fabricant Prodotto da Fabricado por Producent Tillverkare Κατασκευαστής	Authorised representative Bevollmächtigter Representante autorizado Mandataire Rappresentanza autorizzata Representante autorizado Leverandør Auktoriserad representant Εξουσιοδοτημένος αντιπρόσωπος