CE BIOPHEN Plasminogen (LRT)
Ref 221511

Chromogenic assay for measuring Plasminogen activity in plasma, with ready to use liquid reagents

For in vitro diagnostic use only

HYPHEN BioMed

155, rue d'Eragny, F 95000 Neuville-sur-Oise

CoaChrom Diagnostica GmbH

www.coachrom.com | info@coachrom.com

Tel. +43-1-236 222 1 | Fax +43-1-236 222 111

Kostenfreie Nummern für Deutschland:

Tel. 0800-24 66 33-0 | Fax 0800-24 66 33-3

INTENDED USE:

BIOPHEN Plasminogen (LRT) kit is a chromogenic assay for the quantitative determination of Plasminogen Activity in human plasma, using a manual or an automated method. This assay is characterized by that all the reagents are in the liquid presentation, ready to use
(LRT = Liquid reagent Technology).

CLINICAL APPLICATIONS:

Assay for Plasminogen quantitation in human plasma for the diagnosis of congenital or acquired Plasminogen deficiencies. An abnormal Plasminogen activity is an indicator for fibrinolytic troubles.

ASSAY PRINCIPLE:

Plasminogen (Plg) is the plasma precursor for the fibrinolytic enzyme plasmin, which is generated following plasminogen activation by specific biological activators such uPA and tPA, or pharmacological activators such as streptokinase.

Using the BIOPHEN Plasminogen (LRT) assay, Plasminogen is measured following its specific activation by addition of streptokinase and plasminogen-free fibrinogen in excess. The complex plasminogen-streptokinase formed has a "plasmin-like" activity, which then specifically cleaves the plasmin-specific substrate SPm41, releasing para-nitroaniline (pNA), which colour is measured at 405nm. There is a direct relationship between colour development and Plasminogen activity in the tested plasma.

Plg + Streptokinase ⟶ [Plg-Streptokinase]

[Plg-Streptokinase]
SPm41 ⟶ Peptide + pNA

REAGENTS:

R1: *Reagent 1:* Streptokinase.
Activation reagent containing streptokinase (about 15,000 IU/ml) and plasminogen-free fibrinogen derivatives, liquid form:
3 vials of 3 ml each, ready to use.

R2: *Reagent 2:* Substrate
Chromogenic substrate, specific for plasmin and "plasminogen-streptokinase" complexes (SPm41), liquid form:
3 vials of 3 ml each, at about 2.5 mg/ml, ready to use.

Note:
- All the required cautions must be respected in order to avoid any risk of ingestion or accidental introduction of R1 or R2 in body. In case of skin contact, wash extensively with water. In case of contact with a wound, address to the appropriate medical service, and indicate the biological origin and the nature of the product.
- **Sodium azide** (<1g/L) is used as preservative, and may react with lead and copper plumbing to form highly explosive metal azides. Flush with large volumes of water when discarding into a sink.
- The Streptokinase concentration may present variations from lot to lot, but it is exactly adjusted for each new lot of reagent.
- **Reagents are not interchangeable from lot to lot. Use only reagents from a same kit lot for testing Plasminogen.**

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water, preferably sterile.
- Acetic Acid (20%) or Citric Acid (2%) (End point method).
- Physiological saline (0.9% NaCl) or Imidazole Buffer (ref AR021)
- Calibration plasma (ex: **BIOPHEN Plasma Calibrator Ref 222101**) and quality control plasmas (ex: **BIOPHEN Normal Control Plasma Ref 223201**, and **BIOPHEN Abnormal Control Plasma Ref 223301**), titrated for plasminogen activity.

Material:

- Spectrophotometer, photometer or automates for chromogenic assays, with a wavelength set up at 405 nm.
- Stop watch.
- Calibrated pipettes.

STORAGE CONDITIONS:

Reagents must be stored at 2-8°C, in their original packaging box. They are then stable until the expiration date printed on the box.

Note: Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.

PREPARATION AND STABILITY OF REAGENTS:

Note: Refer to each specific instrument adaptation.

R1: Reagent 1: Streptokinase
Ready to use. Incubate at room temperature (18-25°C) for 30 minutes, while shaking the vial from time to time. Homogeneize the content before each use.

Stability of R1, kept in its original vial, and provided that any contamination or evaporation is avoided during use:
- 1 month at 2-8°C.
- 7 days at Room Temperature (18-25°C).

R2: Reagent 2: Plasmin specific chromogenic substrate (SPm41)
Ready to use reagent. Incubate at room temperature (18-25°C) for 30 minutes, while shaking the vial from time to time. Homogeneize the content before each use.

Stability of substrate, kept in its original vial, and provided that any contamination or evaporation is avoided during use:
- 1 month at 2-8°C.
- 7 days at Room Temperature (18-25°C).

Cautions:
- In order to improve stability, reagents must be closed with their original screw cap following each use (white cap for streptokinase (R1), yellow cap for substrate (R2).
- Reagents must be handled with care, in order to avoid any contamination during use.
- The substrate is slightly yellow. If the substrate becomes very yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- Incubating the vials, for 30 min. at RT, allows stabilising the reagents, and obtaining a homogeneous reactivity over time.
- In order to prevent evaporation of reagents, limit the exchange surface by using, for example, a vial neck or an operculated cap.

Note:
- According to the automated method used: in any case, the established reactive ratios, between R1 and R2 (respective reagent concentrations in the reactive milieu), must be followed.
- Do not mix reagents from kits with different lots when running the assay. Reagents R1 and R2 are optimized for each lot of kits.

PREPARATION OF PLASMA:

- Sample collection :

Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), through a net venipuncture with great care, in order to avoid any activation.

- Centrifugation :

The centrifugation step is important and is intended to separate the plasma from the platelets. This must be performed quickly after blood collection using citrate tubes or CTAD (Citrate, Theophylline, Adénosine and Dipyridamole) tubes.
Use a validated method established by your laboratory to obtain platelet poor plasma. For example, 15 minutes at 2000g at room temperature (18-25°C). Use nonactivating plastic tubes and pipettes for handling and storage.

- Plasma Storage :

2 hours at room temperature (18-25°C), 24 hours at 2-8°C and > 2 months frozen at >-20°C

Note : Refer to GEHT and/or NCCLS/CLSI document H21-A5 for further instructions on specimen collection, handling and storage.

TEST PROCEDURE:

BIOPHEN Plasminogen (LRT) kit is designed for being used with kinetics methods, automated, but it can also be used for end point manual methods. Adaptations to the various automates are available upon request. The assay is performed at the controlled temperature of **37°C** and the colour development is measured at **405 nm.**

CALIBRATION:

Calibration is performed with a normal pooled human citrated plasma (made with plasmas from at least 30 normal individuals, males or females, aged between 18 and 55 years, and free of any medication or disease), with the assigned value of **100%** Plasminogen. The assay includes a standard plasma dilution of **1:30**. By definition, this latter dilution of the pool represents the **100 %** Plasminogen activity. The dynamic range is from **0 to 150 %** Plasminogen. The **150 %** Plasminogen activity is then the **1:20** dilution of the **plasma pool** (in physiological saline).
(**Or** calibration can also be performed with a commercially available plasma calibrator, with a known Plasminogen concentration **C**. The **1:30** dilution corresponds to the indicated Plasminogen concentration. The **150%** Plasminogen concentration is obtained (in the assay conditions) by using the following dilution factor: **20 x C :100**).
The calibration curve can then be prepared as follows from the preparation already adjusted at 150% plasminogen:

% Plasminogen	"150% Plasminogen Calibrator" (µL)	Physiological saline (µL)
0	0	500
37.5	125	375
75	250	250
100 (if required)	333	167
150	500	0

In order to get the full assay performances, the calibration curve must be prepared just before running the assay.

ASSAY PROTOCOL:

Manual Method:

Tested plasmas and controls are assayed at the **1:30** dilution in physiological saline.

In a microplate well, or in a **plastic** tube preincubated at **37°C**, introduce:

Reagents	Microplate	Test Tube
Calibrators, or diluted tested plasmas or controls	50µL	200 µL
R1 : Streptokinase preincubated **at 37°C**	50µL	200 µL
Mix and Incubate for **3** min at 37°C, then introduce:		
R2: Substrate preincubated **at 37°C**	50µL	200 µL
Mix and Incubate for **3** min at 37°C, exactly		
Stop the reaction by introducing:		
Citric Acid (20g/L)	50µL	200 µL
Mix and measure the optical density at **405nm** against the sample blank.		

The yellow colour obtained is stable for 2 hours.

The sample blank is obtained by mixing the reagents in the opposite order from that of the test i.e.: Citric Acid (20 g/L), Substrate, Streptokinase, diluted sample.
Measure the Absorbance at 405 nm (A405). Substract the sample blank from the A405 obtained for the assay.

Automated methods:

Adaptations to the various analysers are available upon request. The assay is then performed kinetically. The reaction does not require to be stopped and sample blanks are automatically subtracted. **Refer to each specific adaptation and specific cautions for each instrument.**

NB:
- If higher or lower reactive volumes than those indicated here above are required for the method used, the same respective proportions between reagent concentrations and volumes used, must be adhered to, in order to maintain the assay performance.
- Run a sample blank in presence of highly lipemic, icteric or haemolysed plasmas, or if the plasmas has a "colour" different from the usual one.

QUALITY CONTROL:

Using commercially available quality control plasmas, titrated for Plasminogen activity, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents. The calibration curve is acceptable when the concentrations measured for controls are within the acceptance range. Various control plasmas are available: **BIOPHEN Normal Control Plasma (#223201)** and **BIOPHEN Abnormal Control Plasma (#223301).** Each laboratory should verify its own target value and acceptance range, in the exact working conditions, for each new lot of controls.

Note:
A new calibration curve must be carried out for each new lot of reagents, after each important maintenance of the analyzer, or when measured values for the quality controls are out of the acceptance range determined for the method. Each laboratory can establish its own acceptance ranges, according to the instruments and protocols used. At least one quality control (at different levels) should be include in each test series.

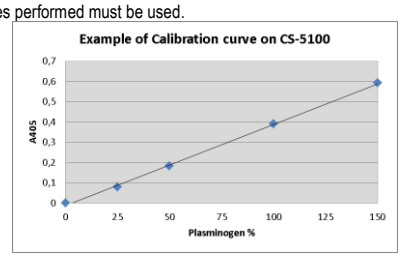
RESULTS:

- For the end point method, using a **linear** graph paper, plot on abscissa the Plasminogen concentration (**%**) and on ordinates the corresponding absorbance (**A405**). Alternatively, statistics software can be used for establishing the dose response calibration curve. A linear relationship is obtained between Plasminogen concentrations and Absorbances (A405).
- Draw the calibration curve obtained. Calculate the "r^2" value. Calibration is acceptable if: **$r^2 \geq 0.98$**, and if measured values for controls are in compliance.
  Usually, using the manual method (test tube), the A405 values range from about 0 for the 0% Plasminogen concentration, to about 2.50 (2.50 ± 0.50) for the 150% Plasminogen concentration. Indicatively, for the microplate method, A405 is expected lower than using the test tube method. A405 values can differ according to the instrument application used.
- The Plasminogen concentration in the tested sample is directly obtained on the calibration curve. Results are expressed as % of Plasminogen.
- Using automated methods, the Plasminogen concentrations are directly calculated by the analyzer, respectively to the calibration curve.
- The dynamic range is from 10 to 150 %; the assay being linear up to 150% Plasminogen activity.

When the assay dilution is **1:30**, the Plasminogen concentration is directly read on the calibration curve. When different dilutions are used, the results must be multiplied by the dilution factor "**D**", divided by **30**, i.e. **D:30.**

EXAMPLE OF CALIBRATION CURVE:

The calibration curve below is displayed as an example only. Only the calibration curve generated for the series of measures performed must be used.



PERFORMANCES AND CHARACTERISTICS:

- The detection threshold is calculated from the calibration curve by measuring the "apparent" concentration which corresponds to the average A405 observed for a Plasminogen deficient sample plus 2 standard deviations (SD). This detection threshold is ≤ 10%.
- Example of Intra-Assay and Inter-Assay reproducibilities obtained on CS-5100 instrument for samples with variable Plasminogen concentrations :

Samples	Plasminogen concentrations %	Intra-Assay			Inter-Assay		
		N	SD	CV %	N	SD	CV %
Sample 1	88	10	0.55	0.6	10	1.0	1.1
Sample 2	29	10	0.27	0.9	10	0.5	1.8

- Correlation: BIOPHEN Plasminogen LRT assay shows good correlation on CS-5100, with:
  BIOPHEN Plasminogen : n=40 r = 0.999
  BERICHROM plasminogen : n=40 r = 0.993

LIMITATIONS OF THE PROCEDURE:

- No significant interference is observed (using CS instrument) for heparin concentrations < 2 IU/mL, bilirubin concentrations <0.25 mg/dl, triglyceride concentration <300mg/dl and haemoglobin concentrations < 500 mg/dl in plasma.
- No significant interference of plasma fibrinogen concentration in the assay.
- In order to get the optimal performances of the assay, the procedural instructions must be strictly respected.

EXPECTED VALUES:

By definition, the 100 % Plasminogen concentration corresponds to the concentration in a normal human citrated plasma pool, obtained by pooling plasmas from healthy males or females aged from 18 to 55 years, and out of any medication, diluted 1:30.
The Plasminogen concentration in healthy adults is usually in the range 60 to 140% (normal range was determined at about 70-130% (mean±2SD) using the BIOPHEN Plasminogen LRT assay, with manual method, on n=54 healthy individuals).
Plasminogen concentration is low in neonates. In healthy adults plasminogen level variations are observed depending on various factor (age, smoking habits, pregnancy, hormonal contraceptives,..).

CLINICAL VARIATIONS:

Plasminogen concentration ≤ 50% (in adults) indicates the presence of a deficiency, which must be confirmed with another test and / or by testing another plasma sample from the patient.
Plasminogen deficiencies can be:
- Mostly acquired: they have been observed in hepatic diseases, DIC, sepsis, thrombolytic therapy using plasminogen activators… and in clinical situations associated with hyperfibrinolytic conditions.
- Hereditary: they can be of type I (hypoplasminogenemia, reduced activity and antigen levels) or of type II (dysplasminogenemia, decreased activity, but normal for antigen level). They could then be associated with an increased thrombotic risk, still discussed.

Ligneous conjunctivitis could also represent a rare but serious complication related to plasminogen deficiency.

BIOCHEMISTRY:

Plasminogen is a single chain glycoprotein of about 90KDa, synthesized in particular in the liver, and usually present at about 200µg/ml in plasma.

Major component of the fibrinolytic system, the plasminogen zymogen is converted to plasmin following partial cleavage by specific activators. Plasmin proteolytic activity is mainly targeted towards fibrin (clot lysis), plasminogen being activated into plasmin around the fibrin clot surface in physiological conditions.
Regulation is ensured by various endogenous activators (uPA, tPA,…) or inhibitors (PAI-1). Exogenous streptokinase also acts as an activator.
The major physiological inhibitor of plasmin in blood is the fast acting α2 plasmin inhibitor.

REFERENCES:

1. Okamoto A, Sakata T, Mannami T, Baba S, Katayama Y, Matsuo H, Yakasa M, Minematsu K, Tomoike H, Miyata T, « Population-based distribution of plasminogen activity and estimated prevalence and relevance to thrombotic diseases of plasminogen deficiency in the Japanese: the Suita study », *J. Thromb Haemost*., 1:2397-2403, 2003.
2. Duboscq C, Quintana I, Bassilotta E, Bergonzelli GE, Porterie P, Sassetti B, Haedo AS, Wainsztein N, Kruithof EK, Kordich L, "Plasminogen: an important parameter in septic patients", *Thromb Haemost*., 77(6): 1090-1095, 1997.
3. Azuma H, Uno Y, Shigekiyo T, Saito S, « Congenital plasminogen deficiency caused by a Ser^{572} to Pro mutation », *Blood*, 82(2):475-480, 1993.
4. Tait RC, Walker ID, Conkie JA, Islam SI, McCall F, Mitchell R, Davidson JF, "Plasminogen levels in healthy volunteers - influence of age, sex, smoking and oral contraceptives", *Thromb Haemost*., 68(5):506-510, 1992.
5. Ponting CP, Marshall JM, Cederholm-Williams SA, "Plasminogen: a structural review", *Blood Coagul Fibrinolysis*, 3(5):605-614, 1992.
6. Schutta HS, Williams EC, Baranski BG, Sutula TP, « Cerebral venous thrombosis with plasminogen deficiency », *Stroke*, 22:401-405, 1991.
7. Aoki N, Moroi M, Sakata Y, Yoshida N, Matsuda M, "Abnormal Plasminogen: a hereditary molecular abnormality found in a patient with recurrent thrombosis", J. Clin. Invest., 1977, 1186-1195, 1977.
8. Reddy KNN, Markus G, "Mechanism of activation of human plasminogen by streptokinase", *J. Biol. Chem*., 247(6):1683-1691, 1972.
9. http://www.ncbi.nlm.nih.gov; OMIM; "Plasminogen, Plasminogen deficiency" (+173350).