

# BIOPHEN Plasminogen

Ref A221502-RUO

Chromogenic assay for measuring Plasminogen activity in plasma

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**



Manufactured By: HYPHEN BioMed

Last revision: 31/03/2011

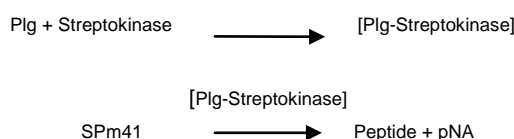
## INTENDED USE:

BIOPHEN Plasminogen kit is a chromogenic assay for the quantitative determination of Plasminogen Activity in human plasma, using a manual or an automated method. **This kit is for research use only and should not be used for patient diagnosis or treatment.**

## ASSAY PRINCIPLE:

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

Using the BIOPHEN Plasminogen assay, Plasminogen is measured following its specific activation by addition of streptokinase and plasminogen-free fibrinogen in excess. The complex formed between plasminogen and streptokinase possesses 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.



## REAGENTS:

### R1: Reagent 1: Streptokinase.

Activation reagent containing streptokinase (about 25,000 IU) and plasminogen-free fibrinogen, lyophilized and stabilized.

2 vials (to be reconstituted with 2.5 mL of distilled water).

### R2: Reagent 2: Substrate

Chromogenic substrate, specific for plasmin and "plasminogen-streptokinase" complexes (SPM41), lyophilized:

2 vials of about 6.25 mg (to be reconstituted with 2.5 mL of distilled water).

**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.

## REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

### Reagents:

- Distilled water, preferentially sterile.
- Acetic Acid (20%) or Citric Acid (2%) (End point method).
- Physiological saline.
- Calibration (ex: **BIOPHEN Plasma Calibrator Ref A222101**) and quality control plasmas (ex: **BIOPHEN Normal Control Plasma Ref A223201**, and **BIOPHEN Abnormal Control Plasma Ref A223301**), 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:

BIOPHEN Plasminogen 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.

## PREPARATION AND STABILITY OF REAGENTS:

### R1: Reagent 1: Streptokinase

- Reconstitute each vial with 2.5 mL of distilled water. Shake thoroughly until complete dissolution of the content (vortex).
- Incubate at room temperature (18-25°C) for 30 minutes, while shaking the vial from time to time.
- Homogenize the content before each use.

Stability of reconstituted R1, kept in its original vial:

- 1 month at 2-8°C.
- 7 days at Room Temperature.
- Do not freeze.

### R2: Reagent 2: Plasmin specific chromogenic substrate (SPM41)

- Reconstitute each vial with 2.5 mL of distilled water. Shake thoroughly until complete dissolution of the content (vortex).
- Incubate at room temperature (18-25°C) for 30 minutes, while shaking the vial from time to time.
- Homogenize the content before each use.

Stability of restored substrate, kept in its original vial:

- 1 month at 2-8°C.
- 7 days at Room Temperature.
- Do not freeze.

### 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 SPM41 (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.

### Note:

- R1 and R2 are closed under vacuum. Remove carefully the stopper, in order to avoid any lost of powder when opening the vials.
- According to the automated method used, the reagents can be reconstituted with volumes different from those recommended. In any case, the established reactive ratios, between R1 and R2 (respective reagent concentrations in the reactive milieu), must be adhered to.
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay. Reagents R1 and R2 are optimized for each lot of kits.
- The stability studies at 30°C show that the reagents can be shipped at room temperature without damage.

## PREPARATION OF PLASMA:

Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), with great care, in a silicon glass or a plastic tube. Sampling must be performed through a net venipuncture, avoiding any blood activation.

- Within 4 hours, blood must be centrifuged at 3,000 g for 20 min at 18°C or below, and plasma decanted into a plastic tube, using a plastic pipette.

Refer to GEHT or NCCLS guidelines for further instructions on specimen collection, handling and storage.

## TEST PROCEDURE:

BIOPHEN Plasminogen 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
150	500	0

D.750.02/BI/1502/RUO



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

## 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
<b>R1 : Streptokinase preincubated at 37°C</b>	50µL	200 µL
Mix and Incubate for 3 min at 37°C, then introduce:		
<b>R2: Substrate preincubated at 37°C</b>	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 <b>405nm</b> 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). Subtract 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.

#### 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:

The control is performed using commercially available control plasmas, titrated for Plasminogen activity. Various control plasmas are available:

**BIOPHEN Normal Control Plasma: (ref A223201).**

**BIOPHEN Abnormal Control Plasma: (ref A223301).**

Use of quality control plasmas allows validating the calibration curve, as well as the homogeneous reactivity of the BIOPHEN Plasminogen assay from run to run, and from series to series, when using a same lot of reagents.

## LIMITATIONS OF THE PROCEDURE:

- No significant interference is observed (using STA) for heparin concentrations < 2 IU/mL, bilirubin concentrations < 0.2 mg/ml, and haemoglobin concentrations < 2 mg/ml 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.
- **The results obtained should be for research purposes only and not used for patient diagnosis or treatment.**

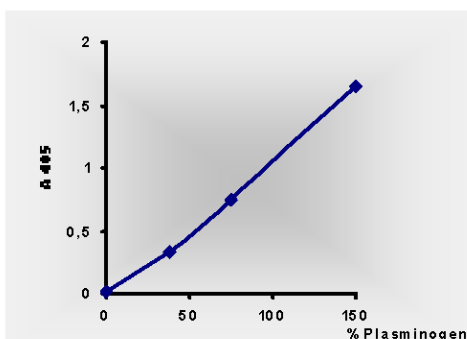
## RESULTS:

- For the end point method, using a linear graph paper, plot on abscissa the Plasminogen concentration (%) and on ordinates the corresponding absorbance (**A405**). 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 indicated as an example only. Only the calibration curve generated for the series of measures performed must be used.



## VALIDATION OF CALIBRATION CURVE:

The calibration curve is acceptable when the concentrations measured for the Control Plasmas are within the acceptance range.

## PERFORMANCES AND CHARACTERISTICS:

- The detection threshold is calculated by measuring the "apparent" A405 obtained for a Plasminogen deficient sample plus 3 standard deviations (SD). This detection threshold is  $\leq 10\%$ .
- Example of Intra-Assay and Inter-Assay reproducibilities obtained for samples with variable Plasminogen concentrations (manual method) :

Samples	Plasminogen concentrations %	Intra-Assay CV%	N	Inter-Assay CV%	N
Sample 1	101	0.99	10	6.7	8
Sample 2	55	2.01	10	5.0	8

## 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 in the fibrinolytic system, 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 to plasmin onto the fibrin clot 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  $\alpha 2$  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.) Dubocq C, Quintana I, Bassilotta E, Bergonzelli GE, Porterie P, Sasseti B, Haedo AS, Wainsztein N, Kruihof 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<sup>572</sup> 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).