HYPHEN BioMed

155 rue d'Eragny, 95000 Neuville-sur-Oise, France
Tél : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
www.hyphen-biomed.com
info@hyphen-biomed.com

IVD

BIOPHEN™ Plasminogen LRT

REF 221511

R1 R2 3 x 3 mL

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

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INTENDED USE:

The BIOPHEN™ Plasminogen LRT kit is a chromogenic method for *in vitro* quantitative determination of Plasminogen activity on citrated human plasma, using a manual or automated method. Reagents are in the liquid presentation, ready to use (LRT, Liquid reagent Technology).

SUMMARY AND EXPLANATION:

Technical :[5,8,13]

Major component of the fibrinolytic system, Plasminogen (Plg) zymogen is converted to plasmin by specific activators and functional activity measured by chromogenic assay. Plasmin proteolytic activity is mainly targeted towards fibrin (clot lysis), Plg being activated into plasmin around the fibrin clot surface in physiological conditions. Regulation is ensured by activators (eg endogenous uPA, tPA or exogenous streptokinase) and inhibitors (eg PAI1, α2 antiplasmin).

Clinical :[1-4,6-7,10-13]

Measuring Plasminogen activity in human plasma is used for help in the diagnosis of congenital or acquired Plg deficiencies.

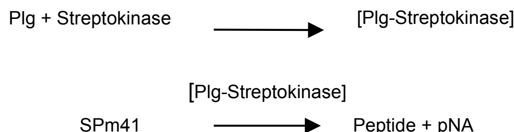
Acquired deficiencies are observed eg in liver disease, DIC, sepsis, thrombolytic therapy using plasminogen activators, hyperfibrinolytic contexts…

Congenital deficiencies can be of Type I (quantitative) or Type II (qualitative) and could be associated with increased thrombotic risk, still discussed.

Ligneous conjunctivitis and lesions could represent complications related to plasminogen deficiency. An abnormal Plg activity is an indicator for fibrinolytic troubles. Plasminogen concentration is decreased in neonates.

PRINCIPLE:

Using the BIOPHEN™ Plasminogen LRT assay, Plasminogen in plasma is measured following a specific activation with streptokinase and Plg free fibrinogen derivatives in excess. The formed "plasmin-like" activity complex hydrolysis the chromogenic substrate (SPm-41) which releases paranitroaniline (pNA). The amount of pNA released (measured by absorbance at 405 nm) is directly proportional to the concentration of Plg in the specimen.



REAGENTS:

R1 **Streptokinase** : Activation reagent containing streptokinase (about 15 000 IU/mL) and plasminogen-free fibrinogen derivatives, stabilized, liquid form. Contains BSA.

R2 **Chromogenic substrate specific for Plasmin and « plasminogen-streptokinase » complexes (SPm41),** at about 2.5 mg/mL, stabilized, liquid form. Contains Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H -isothiazol-3-one (3:1).

R1 R2 3 vials of 3 mL.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R1 R2 Reagent is ready to use; homogenize, avoiding formation of foam, and load it directly on the analyzer following application guide instruction.

For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R1 R2 Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **1 month** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Stability on board of the analyzer: see the specific application.**

If the substrate becomes yellow, this indicate a contamination. Discard the vial and use a new one.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Diluent: Physiological Saline (0.9% NaCl) or Imidazole buffer (AR021K/AR021L/AR021B/AR021M/AR021N). Use the same buffer for all dilutions performed.
- Specific calibrators and controls with known titration, such as:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301

Also refer to the specific application guide of the analyzer used.

Materials:

- Spectrophotometer or analyzer for chromogenic assays.
- Stopwatch; Calibrated pipettes; plastic test tubes or microplate.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[9] guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references[9].

PROCEDURE:

The kit can be used for kinetics, automated or manual (endpoint) methods. Perform the test at **37°C** and read color intensity at **405nm.**

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay method:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. For the calibration curve, dilute the calibrators in physiological saline as described below ("C" defines the concentration of Plasminogen).

The **1:30** dilution corresponds to the indicated concentration (**C**) of Plasminogen and the **1:20** dilution to 1.5 fold this concentration (3C:2).

Prepare **2 mL** of the **150%** Plasminogen concentration (C1) (in the assay condition) by using a (**20xC/100**) dilution factor.

The calibration range can then be prepared as follows:

Plasminogen	C1	C2	C3	C4	0
% Plasminogen	150	100	75	37.5	0
Volume of Calibrator at 150%	500µL	333µL	250µL	125µL	0µL
Volume of physiological saline	0µL	167µL	250µL	375µL	500µL

2. Dilute the specimens and controls in physiological saline, as described in the table below:

Specimens	Reference	Dilution
Controls	223201 / 223301	**1:30**
Specimens	N.A.	**1:30**

Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. Dispense the following to the wells of a microplate, or to a plastic tube incubated at 37°C:

	Microplate	Test tube
Specimens, calibrators or controls diluted	50 µL	200 µL
R1 **Streptokinase** Pre-incubated at **37°C**	50 µL	200 µL
Mix and incubate **at 37°C for 3 minutes**, then add the following:		
R2 **Chromogenic substrate** Pre-incubated at **37°C**	50 µL	200 µL
Mix and incubate at **37°C** for **3 minutes exactly**		
Stop the reaction by adding:		
Citric acid (2%)*	50 µL	200 µL
Mix and measure the optical density at **405nm** against the corresponding blank.		

*Or acetic acid (20%). The yellow color is stable for 2 hours.
The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Citric acid (2%), R2, R1, dilute specimen**.**
Measure the optical density at 405 nm. Subtract the measured blank value from the absorbance measured for the corresponding test.

Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

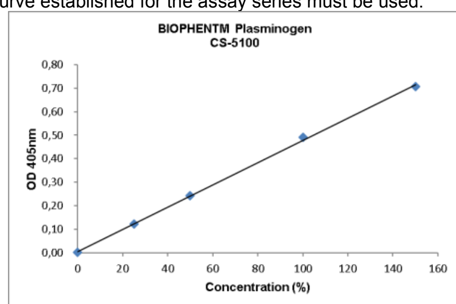
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ Plasminogen LRT assay can be calibrated for the assay of Plasminogen activity. The calibrator covering the calibration range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

- The calibration range is about 0 to 150% (on CS-series).

The calibration curve shown below is given by way of example only. The calibration curve established for the assay series must be used.



QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve lin-lin, with the OD 405 nm along the Y-axis and the Plasminogen concentration, expressed as %, along the X-axis.
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- The concentration of Plasminogen (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- The results should be interpreted according to the patient's clinical and biological condition.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- An unexpected abnormal result should be confirmed by another method and/or another sample collected, and considered according to the clinical context.

EXPECTED VALUES:

The Plasminogen concentration in adults is usually expected between 80% and 140% from internal data and literature (variable according to age, ethnicity, smoking, pregnancy, contraceptives, ….).[1,3,4,10,11] However, each laboratory has to determine its own normal range.

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used (<2% on Sysmex CS-5100).
- The measuring range depends on the analytical system used (about 2 to 150% of Plasminogen on Sysmex CS-series).
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 2 repetitions within each series for a control level. The following results were obtained:

Control	Intra assay				Inter assays			
	n	Mean%	CV%	SD	n	Mean%	CV%	SD
Control 1	10	91.3	0.8	0.8	20	91.5	1.1	1.0
Control 2	10	29.1	1.3	0.4	20	29.1	1.8	0.5

- Correlation with reference method (Berichrom Plasminogen vs BIOPHEN™ Plasminogen LRT on Sysmex CS-5100):
  n = 63 y = 1.11x – 14.20 r = 0.995
- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	Bilirubin (C/F)	Intralipids	Heparins (UFH/LMWH)
500 mg/dL	28 mg/dL	300 mg/dL	2 IU/mL

No significant interference of plasma fibrinogen concentration in the assay.
Also refer to the specific application guide of the analyzer used.

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SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

R2 H317 : May cause an allergic skin reaction.