

BIOPHEN™ Protein C LRT

REF 221211-RUO

R1 **R2** 3 x 3 mL

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

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

English, last revision: 01-2023

INTENDED USE:

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

This kit is for research use only and must not be used for patient diagnosis or treatment.

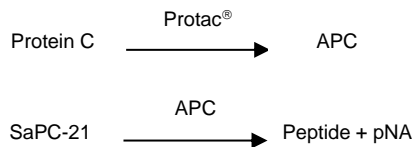
SUMMARY AND EXPLANATION:

Technical:

Protein C is a glycoprotein, vitamin K dependent, which inhibits coagulation. Its normal concentration in human plasma is about 4 µg/mL. Activated by the thrombomodulin-thrombin complex, the activated Protein C (APC), in presence of its cofactor the Protein S, calcium and phospholipids (PPL), will cleave Factors Va and VIIIa, suppressing their procoagulant cofactor activity¹.

PRINCIPLE:

Using the BIOPHEN™ Protein C LRT assay, Protein C in plasma is measured following a specific activation with Protac®, an enzyme extracted from snake venom (Agkistrodom C Contortrix). Activated Protein C (APC) hydrolysis the specific chromogenic substrate (SaPC-21) which releases para-nitroaniline (pNA). The amount of pNA released (measured by absorbance at 405 nm) is directly proportional to the concentration of Protein C in the specimen.



REAGENTS:

R1 **Protac®**. Highly purified enzyme, extracted from the Agkistrodom C Contortrix snake venom, stabilized, liquid form, able to specifically activate Protein C. Each vial contains about 0.32 U/mL of Protac®. Contains BSA.

R2 **SaPC-21**. Chromogenic substrate, specific for Activated Protein C, stabilized, liquid form. Each vial contains about 1.6 mg/mL of SaPC-21. Contains a mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1) and Cesium Chloride.

R1 **R2** 3 vials of 3 mL.

The Protac® concentration may present variations from lot to lot, but it is exactly adjusted for each new lot of reagent.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. 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.
- All the required cautions must be respected in order to avoid any risk of ingestion or accidental introduction of **R1** (Protac®) 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.
- Please consult Safety Data Sheet (SDS), available on www.hyphen-biomed.com.
- R2** (SaPC-21) - Skin sensitizer (Cat 1, H317), Reprotoxic (Cat 2, H361f)
 - P201: Obtain special instructions before use.
 - P280: Wear protective gloves/protective clothing/eye protection/face protection.
 - P302+P352: IF ON SKIN: Wash with plenty of soap and water.
 - P308+P313: IF exposed or concerned: Get medical advice/attention.

- P333+P313: If skin irritation or rash occurs: Get medical advice/attention.
- P405: Store locked up.
- P501: Dispose of contents/container in consultation with your regional waste disposer.
- 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* 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:
- 5 weeks at 2-8°C.
 - 7 days at room temperature (18-25°C).
 - Do not freeze.
 - 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).
- Physiological Saline (0.9% NaCl).
- Specific calibrators and controls with known titration, such as:

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

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

Materials:

- Spectrophotometer or automatic instrument for chromogenic assays.
- Stopwatch; Calibrated pipettes; Plastic 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-A5³ guideline for further information concerning specimen collection, handling and storage). For plasma storage, please refer to references^{2,3,4}.

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 Protein C). The 1:2 dilution corresponds to the indicated concentration (C) of PC and the 3:4 dilution to 1.5 fold this concentration (3C:2).

The calibration range can then be prepared as follows:

% Protein C	3C:2	C	C:2	C:4	0
% Protein C	150	100	50	25	0
Volume calibrator	150µL	250µL	250µL	125µL	0µL
Volume Physiological Saline	50µL	250µL	750µL	875µL	1000µL

2. Dilute the specimens and controls in Physiological Saline, as described in the table below:

Specimens	Reference	Dilution
Controls	223201-RUO / 223301-RUO	1:2
Specimen	n.a.	1:2

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	Volume
Diluted specimens, calibrators or controls	25 µL	50 µL
R1 Protac® Pre-incubated at 37°C	100 µL	200 µL
Mix and incubate at 37°C for 5 minutes, then add the following:		
R2 SaPC-21 Pre-incubated at 37°C	100 µL	200 µL
Mix and incubate at 37°C for 5 minutes exactly		
Stop the reaction by adding:		
Citric acid (2%)*	100 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.

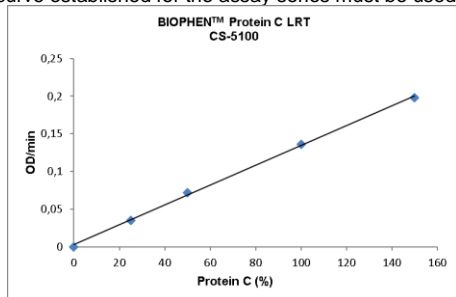
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ Protein C LRT assay can be calibrated for the assay of Protein C 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%.

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 Protein C concentration, expressed as %, along the X-axis. When employing the kinetics method, use ΔOD 405 instead of OD 405.
 - The concentration of Protein C (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- The results obtained should be for research use only and must not be used for patient diagnosis or treatment.**

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.
- Presence of anti-human Protein C antibodies in plasma may inhibit activated Protein C amidolytic activity when performing the assay.

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used (<1.8% on Sysmex CS-5100).
- The measuring range depends on the analytical system used (about 7 to 200% of Protein C on Sysmex CS-series).
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 20-day period, 2 series per day and 3 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	40	35.7	2.2	0.8	120	36.3	2.4	0.9
Control 2	40	81.2	1.5	1.2	120	84.1	2.0	1.7

- By the assay principle, no interference of anti-Xa and anti-IIa anticoagulants, such as Rivaroxaban, Apixaban, Edoxaban, Dabigatran or UFH, is expected.
- Correlation with reference method (Berichrom Protein C Kit (Siemens) vs BIOPHEN™ Protein C LRT on Sysmex CS-5100) :
 $n = 114$ $y = 1.04x - 4.04$ $r = 0.979$

Interferences:

No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	Bilirubin (F/C)	Intralipids
500 mg/dL	60 mg/dL	1000 mg/dL

Refer to the specific application guide of the analyzer used.

REFERENCES:

- Stenflo J.: Structure and Function of Protein C. Semin. Thromb. Haemostasis. 1984.
- CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
- Woodhams B. et al. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.
- Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.

SYMBOLS:

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

- R2** H317: May cause an allergic skin reaction.
- H361f: Suspected of damaging fertility.

Changes compared to the previous version.