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.

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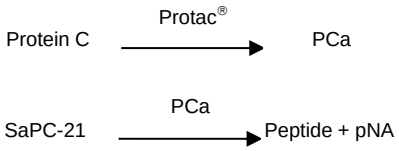
INTENDED USE:

The BIOPHEN™ Protein C (LRT) kit is a chromogenic method for *in vitro* quantitative determination of Protein C activity on human citrated plasma[1,2] 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.

PRINCIPLE:

Using the BIOPHEN™ Protein C (LRT) assay, Protein C is measured following a specific activation with Protac®, an enzyme extracted from snake venom (Agkistrodom C Contortrix) [3,4]. Activated protein C (APC) then specifically cleaves the specific substrate SaPC-21, releasing para-nitroaniline (pNA), which colour is measured at 405nm. There is a direct relationship between colour development and Protein C activity in the tested plasma.



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.
3 vials of 3 mL.

R2 SaPC-21. Chromogenic substrate, specific for Activated protein C, in liquid form. Each vial contains about 1.6 mg/mL of SaPC-21.
3 vials of 3 mL.

Reagent 1 contains small amounts of sodium azide (0.9 g/L), see WARNINGS AND PRECAUTIONS.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- 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.
- The Protac® concentration may present variations from lot to lot, but it is exactly adjusted for each new lot of reagent.
- A yellow color indicates a contaminated substrate. Discard the vial and use a new one.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits. Do not mix reagents from different kit batches when performing an assay; they are optimized for each batch of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- The bovine plasma used to prepare the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- Create a plasma blank if this latter is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- For *in vitro* use.

R2 H317: May cause an allergic skin reaction.

REAGENT PREPARATION AND STABILITY:

R1 Reagent 1: Protac®

Clear vial, ready to use. Allow to stabilize for 30 minutes at room temperature (18-25°C), before use.
Homogenize the reagent prior to use.
Reagent stability after opening, excluding any contamination or evaporation, and stored in the original vial, is of:

- **5 weeks** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**

R2 Reagent 2: SaPC-21

Brown vial, ready to use. Allow to stabilize for 30 minutes at room temperature (18-25°C), before use.
Homogenize the reagent prior to use.
Reagent stability after opening, excluding any contamination or evaporation, and stored in the original vial, is of:

- **5 weeks** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**

STORAGE CONDITIONS:

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.

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

Materials:

- Spectrophotometer or automatic instrument for chromogenic assays.
- Stopwatch; Calibrated pipettes; Plastic tubes or microplate.

SPECIMEN COLLECTION AND PREPARATION:

Specimens should be prepared and stored in accordance with applicable local guidelines.

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:

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

- Centrifugation:

Within 2 hours, use a laboratory-validated method to obtain platelet-poor plasma, for example at least 15 minutes at 2500 g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.

- Plasma storage[5,6]:
  - 4 hours at room temperature (18-25°C).
  - 1 month at -20°C.
  - 24 months at -70°C.

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

PROCEDURE:

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

Automated methods:

Applications for the various analyzers are available on request. **See the specific application 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):

Calibrator (222101) % Protein C	C	C:2	C:4	0
Volume calibrator	500µL	250µL	125µL	0µL
Volume Physiological Saline	0µL	250µL	375µL	500µL

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

Specimens	Reference	Dilution
Controls	223201-RUO / 223301-RUO	**1:2**
Calibrators (calibration curve)	222101-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 within 1 hour. 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
Specimens, calibrators or controls **diluted 1:2***	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.		

**For the 150% Protein C calibration point, introduce 18.75 µL of undiluted plasma calibrator ("C") and 6.25 µL of physiological saline directly in the micro well or 37.5 µL of same plasma and 12.5 µL of physiological saline directly in the test tube.*

**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.

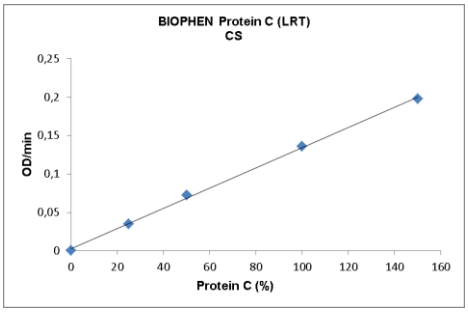
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ Protein C (LRT) assay can be calibrated for the assay of Protein C activity. The plasma calibrator covering the dynamic test 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, obtained on CS-5100 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 defined, 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 acceptable range for the method.

Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve, with the OD 405nm along the Y-axis and the protein C activity, expressed as % of Protein C, along the X-axis.
- The concentration of Protein C in the test specimen is directly inferred from the calibration curve, if the standard dilution is used.
- Results are expressed as % of Protein C.

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. The laboratory is responsible for validating any changes made to these instructions for use.
- 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.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.
- Presence of anti-human Protein C antibodies in plasma may inhibit activated Protein C amidolytic activity when performing the assay.
- For the possible influence of interferences, refer to specific application for the analyzer used (no significant effect is observed on Sysmex CS-5100 for Heparin concentration up to 1 IU/mL, bilirubin concentration up to 60 mg/dL, hemoglobin concentration up to 500 mg/dL and intralipids concentration up to 1000 mg/dL, by plasma overload tests).

PERFORMANCE:

- The lower analyzer detection limit depends on the analytical system used (<1.8% on Sysmex CS-5100).
- On Sysmex CS-series, the measuring range is from about 7 to 200% of Protein C.
- Performance studies were conducted internally on 1 batch of reagent using a Sysmex CS-5100. Performance was assessed using laboratory controls over a 20-day period, 2 series per day and duplicates within each series for a control level. The following results were obtained:

Control	Intra assay				Inter assay			
	n	Mean	CV%	SD	n	Mean	CV%	SD
Control 1	40	35.7	2.2	0.8	80	36.3	2.4	0.9
Control 2	40	81.2	1.5	1.2	80	84.1	2.0	1.7

REFERENCES:

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3. Esmon C.T.: Protein C activation. Semin. Thromb. Haemostasis, 10,2, 122-133, (1984).
4. Axner T. Characterisation and some properties of the Protein C activator from Agkistrodom Concortrix venom. Thromb. Haemostasis 59, 40-44 (1988).
5. Woodhams B. *et al.* Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.
6. 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.

Changes compared to the previous version.

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