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

CE IVD

BIOPHEN™ Protein C 5

REF 221205

R1 R2 4 vials x 5 mL

English, revision: 01-2023

INTENDED USE:

Chromogenic method for the *in vitro* quantitative determination of Protein C activity in human citrated plasma, using a manual or automated method. This method is for the detection of Protein C deficiencies in patients who are suspected of congenital or acquired deficiency.
This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

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 activity[1,2].
Clinical:
Assay of coagulation Protein C in plasma may help in the diagnosis of congenital or acquired Protein C deficiencies[3,4, 5, 6,7,8].
Acquired deficiencies are observed in hepatic diseases, during VKA therapy or in Disseminated Intravascular Coagulation (DIC).
Congenital deficiencies can be quantitative (Type I) or qualitative (Type II) and are associated with recurrent venous thrombosis.
Congenital or acquired Protein C deficiency is a risk factor of venous thrombosis[3].
Protein C activity level varies with age (lower in neonates or children).[8]

PRINCIPLE:

Using the BIOPHEN™ Protein C 5 assay, Protein C in plasma is measured following a specific activation with Protac®, an enzyme extracted from snake venom (Agkistrodom C Contortrix)[4,5]. Activated Protein C (APC) hydrolysis the chromogenic substrate (SaPC-21) which release 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®** at approximately 0.32 U/mL. Highly purified enzyme, extracted from the Agkistrodom Contortrix snake venom, lyophilized and stabilized, able to specifically activate Protein C. Contains BSA and stabilizers.

R2 **SaPC-21** at approximately 1.6 mg/mL. Chromogenic substrate, specific for Activated Protein C, lyophilized. Contains Cesium chloride and stabilizers.
H361f: suspected of damaging fertility.

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

WARNINGS AND PRECAUTIONS:

- This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.
- Please consult Safety Data Sheet (SDS), available on www.hyphen-biomed.com.
- R2 (SaPC-21) - Reprotoxic (Cat 2, H361f)
  - P201: Obtain special instructions before use. .
  - P280: Wear protective gloves/protective clothing/eye protection/ face protection.
  - P308+P313: IF exposed or concerned: 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.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R1 R2 Reconstitute the contents of each vial with exactly **5 mL of distilled water.**
Shake vigorously until complete dissolution while 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 reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **3 months** at 2-8°C.
- **Stability on board of the analyzer: see the specific Application Guide**.

Combination of storage are not recommended.
If the substrate becomes yellow, this indicates a contamination. Discard the vial and use a new one.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Physiological Saline (0.9% NaCl).
- Specific calibrators and controls:

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

- Automatic analyzer for chromogenic assays such as: STA-R®-series.
- Laboratory material.

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. Samples should be collected, prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[10] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[10,11,12].

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**.

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 calibrator in **1:2** in physiological saline to get the C% concentration (by definition 100% for a pool of normal plasma or C% for a commercial calibrator), then prepare the calibration curve as described below ("C" defines the concentration of Protein C):

Calibrator (222101) % Protein C	C	C:2	C:4	0
Volume calibrator **(diluted 1:2)**	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 / 223301	**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
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.		

*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.

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 activity, expressed as %, along the X-axis. When employing the kinetic 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 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 no limpid appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- Aprotinin inhibits Activated Protein C. The "apparent" Protein C activity is decreased in patients treated with aprotinin[9].
- Presence of anti-human Protein C antibodies in plasma may inhibit activated Protein C amidolytic activity when performing the assay.
- User defined modifications are not supported by HYPHEN BioMed as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in HYPHEN BioMed Application Guides or these Instructions for Use.
- An unexpected abnormal result should be confirmed by another method and/or another sample collected, and considered according to the clinical context[6,8].

EXPECTED VALUES:

The normal plasma Protein C level in the adult population is usually in the range of 70 to 140%. However, each laboratory has to determine its own normal range.

PERFORMANCES:

- The following performance data represent typical results and are not to be regarded as specifications for BIOPHEN™ Protein C 5.
- The detection threshold is calculated by measuring the "apparent" A405 obtained for a Protein C deficient sample plus 3 standard deviations (SD). This detection threshold is ≤ 5%.
- The assay working range is from 5 to 140%.
- Example of Intra-Assay and Inter-Assay reproducibilities obtained for samples with variable Protein concentrations:

Samples	Protein C concentrations (%)	Intra-Assay CV(%)	N	Inter-Assay CV(%)	N
1	98	0.37	9	1.26	12
2	59	1.17	10	1.97	12
3	39	0.84	10	1.51	12

- Correlation with reference method (COAMATIC® Protein C vs BIOPHEN™ Protein C 5 on BCS) :
  n = 21 y = 1x + 0.8463 r = 0.998
- Interferences: Refer to the specific application guide of the analyzer used.

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Changes compared to the previous version.

The following symbols may appear on the product labeling:

REF	Catalogue number	LOT	Batch code	IVD	*In-vitro* diagnostic medical device
Rx	Numerical < x> identification of reagent		See instructions for use	WHO STD	WHO standard code
	Temperature limitation		Manufacturer	YYYY-MM-DD	Use by
CE XXXX	CE marking of conformity with notified body ID number.	→	Reconstitution volume	CONTENTS	Contents
Cx	Numerical < x> identification of control	i-MA	See instructions in Method Application guide	CONTAINS	Contains
EXP	Expiration date	Σ	Contains sufficient for <n> tests	UNIT	Measurement unit
TARGET VALUE	Target Value		Keep away from sunlight and heat	CALx	Numerical < x> identification of calibrator
ACCEPTANCE RANGE	Acceptance range				Biological risks

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