


BIOPHEN™ Protein C 5

REF 221205

R1 R2 4 x 5 mL

Chromogenic method for measuring Protein C activity in plasma.

English, last revision: 01-2019

INTENDED USE:

The BIOPHEN™ Protein C 5 kit is a chromogenic method for *in vitro* quantitative determination of Protein C activity on human citrated plasma using manual or automated method.

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 his cofactor the Protein S, calcium and PPL, will cleave Factors Va and VIIIa, suppressing their procoagulant cofactor activity^{1,2}.

Clinical:

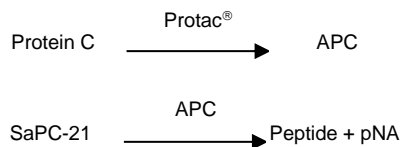
Assay of coagulation Protein C in plasma may help in the diagnosis of congenital or acquired Protein C deficiencies^{3,4,5,6}.

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 thromboses.

Congenital or acquired Protein C deficiency is a risk factor of venous thrombosis³.

PRINCIPLE:

Using the BIOPHEN™ Protein C 5 assay, Protein C in plasma is measured following a specific activation with Protac® (Agkistrodom Contortrix), an enzyme extracted from snake venom^{4,5}. Activated protein C 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®. Highly purified enzyme, extracted from the Agkistrodom Contortrix snake venom, lyophilized and stabilized, able to specifically activate Protein C. Each vial contains about 1.60 U of Protac®. Contains BSA.
4 vials of 5 mL.
R2 SaPC-21. Chromogenic substrate, specific for Activated protein C, lyophilized. Each vial contains about 8 mg of SaPC-21.
4 vials of 5 mL.
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.
- The Protac® concentration may present variations from lot to lot, but it is exactly adjusted for each new lot of reagent.
- 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:

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.
- 3 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
BIOPHEN™ Abnormal Control Plasma	223301
BIOPHEN™ Normal Control Plasma	223201

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-A5⁸ guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references^{8,9,10}.

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.

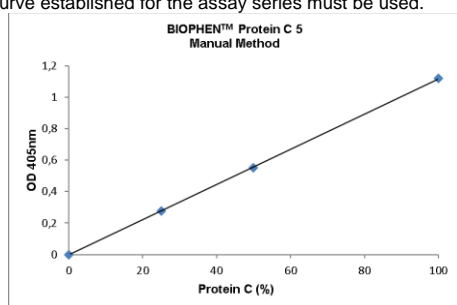
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ Protein C 5 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 100%.

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 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.
- 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.
- Aprotinin inhibits Activated Protein C. The "apparent" Protein C activity is decreased in patients treated with aprotinin⁷.
- Presence of anti-human Protein C antibodies in plasma may inhibit activated Protein C amidolytic activity when performing the assay.

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. The Protein C concentration is decreased in neonates due to hepatic immaturity. It is later independent of age and sex.

A Protein C concentration ≤ 60 % indicates the presence of a deficiency, which must be confirmed by another measurement, and/or another sample collected⁶. Protein C concentration is reduced during VKA therapy, in hepatic diseases, in DIC, and in a congenital deficiency.

PERFORMANCES:

- 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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SYMBOLS:

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

- R1** H315: Causes skin irritation.
 H319: Causes serious eye irritation.
 H335: May cause respiratory irritation

Changes compared to the previous version.