



BIOPHEN Protein C 5 Ref A221205

Chromogenic assay for measuring
Protein C in plasma

For in vitro diagnostic use only



Manufactured By: HYPHEN BioMed

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INTENDED USE:

BIOPHEN Protein C 5 kit is a chromogenic assay for measuring Protein C activity in human citrated plasma^{1,2}, using a manual or automated method.

CLINICAL APPLICATIONS:

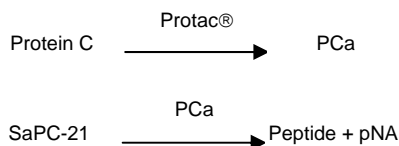
Assay of coagulation Protein C in human plasma for the diagnosis of congenital or acquired Protein C deficiencies^{3,4,5,6}.

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

ASSAY PRINCIPLE:

Protein C is a vitamin K dependent human Protein, which inhibits and regulates coagulation through specific cleavages of Factors Va and VIIIa, suppressing their procoagulant cofactor activity^{1,2}.

Using the BIOPHEN Protein C 5 assay, Protein C is measured following a specific activation with Protac®, an enzyme extracted from snake venom (Agkistrodom C Contortrix)^{4,5}. 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: Reagent 1: Protac, about 1.60 U.

Highly purified enzyme, extracted from the Agkistrodom C Contortrix snake venom, lyophilized and stabilised, able to specifically activate Protein C:

4 vials containing about 1.60 U of Protac® (to be reconstituted with 5 mL of distilled water).

R2: Reagent 2: SaPC-21

Chromogenic substrate, specific for Activated protein C (SaPC-21), lyophilised:

4 vials containing 8 mg of SaPC-21 (to be reconstituted with 5 mL of distilled water).

Note:

- 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.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water, preferentially sterile.
- Acetic Acid (20%) or Citric Acid (2%) (End point method).
- Physiological saline (0.9% NaCl).
- Plasma Calibrator (BIOPHEN Plasma Calibrator Ref A222101).
- Normal or Abnormal Control Plasmas (BIOPHEN Normal Control Plasma Ref A223201, and BIOPHEN Abnormal Control Plasma Ref A223301).

Material:

- Spectrophotometer, photometer or automates for chromogenic assays, with a wave-length set up at 405 nm.
- Stop watch.
- Calibrated pipettes.

STORAGE CONDITIONS:

BIOPHEN Protein C 5 kits must be stored at 2-8°C, in their original packaging box. They are then stable until the expiration date printed on the box.

PREPARATION AND STABILITY OF REAGENTS:

R1: Reagent 1: Protac®

Reconstitute each vial with exactly 5 ml of distilled water. Let the reagent to stabilise for 30 min at Room Temperature, before use.

Shake gently before use.

Stability of reconstituted Protac®, kept in its original vial:

- 3 months at 2-8°C.
- 3 days at Room Temperature.
- Do not freeze.

R2: Reagent 2: Activated Protein C specific Chromogenic substrate (SaPC-21)

Reconstitute each vial with 5 ml of distilled water. Incubate at Room Temperature (18-25°C) for 30 min, before use.

Shake gently before use

Stability of restored substrate, kept in its original vial:

- 3 months at 2-8°C.
- 3 days at Room Temperature.
- Do not freeze.

Cautions:

- In order to improve stability, reagents must be closed with their original screw cap following each use (white caps for Protac®, yellow caps for SaPC-21).
- Reagents must be handled with care, in order to avoid any contamination during use.
- If the substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- To incubate the reconstituted vials, for 30 min. at RT, allows stabilising the reagents, and obtaining a homogeneous reactivity over time.
- In order to avoid evaporation of reagents, limit the exchange surface by using, for example, a vial neck or an operculated cap.

Note:

- R1 and R2 vials are closed under vacuum. Remove carefully the stopper, in order to avoid any lost of powder when opening the vials.
- According to the automated method used, the reagents can be reconstituted with volumes different from those recommended. In any case, the established reactive ratios (respective reagent concentrations in the reactive milieu) between Protac® and Activated Protein C substrate must be strictly respected.
- Use only reagents from kits with a same lot number. Do not mix reagents from kits with different lots when running the assay. Reagents R1 and R2 are optimized for each lot of kits.

PREPARATION OF PLASMA (SPECIMEN COLLECTION):

Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), with great care, in a silicon glass or a plastic tube. Sampling must be performed through a net venipuncture, avoiding any blood activation.

- Within 4 hours, blood must be centrifuged at 3,000 g for 20 min at 18°C or below, and plasma decanted into a plastic tube, using a plastic pipette.
- Storage of plasma:
 - Up to 4 hours at Room Temperature (18-25°C).
 - Up to 24 hours at 2-8°C.
 - Up to 1 month frozen at -20°C or below (before use, thaw for 15 min. in a water bath at 37°C).

Refer to NCCLS document H21-A2 for further instructions on specimen collection, handling and storage.

TEST PROCEDURE:

BIOPHEN Protein C 5 kit is designed for being used in kinetic methods, automated, but it can also be used for end point manual methods. Adaptations for the various automates are available upon request. The assay is performed at the controlled temperature of 37°C and the colour development is measured at 405 nm.

CALIBRATION:

BIOPHEN Protein C 5 kit can be calibrated with the BIOPHEN Plasma Calibrator (ref A222101), which has a well defined Protein concentration, "C". The following calibration range must be prepared as follows:

% Protein C	Plasma Calibrator (µl)	Physiological Saline (µl)
0	0	500
C/4	125	375
C/2	250	250
C	500	0

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ASSAY PROTOCOL:

Manual Method:

Dilute the tested samples, the controls and the calibration solutions 1:2 with physiological saline (0.15 M Sodium Chloride).

In a microplate well, or in a **plastic** tube preincubated at 37°C, introduce:

Reagents	Microplate	Test Tube
Calibrators, Controls or tested plasmas, diluted 1:2	25 µL	50 µL
R1 : Protac® preincubated at 37°C	100 µL	200 µL
Mix and Incubate for 5 min at 37°C, then introduce:		
R2: SaPC-21 Substrate preincubated at 37°C	100 µL	200 µL
Mix and Incubate for 5 min at 37°C, exactly		
Stop the reaction by introducing:		
Citric Acid (20g/L)	100 µL	200 µL
Mix and measure the optical density at 405nm against the sample blank.		

The yellow colour obtained is stable for 2 hours.

The sample blank is obtained by mixing the reagents in the opposite order from that of the test i.e.: Citric Acid (20 g/L), SaPC-21 substrate, Protac®, diluted plasma.

Measure the Absorbance at 405 nm (A405). Subtract the sample blank from the A405 obtained for the assay.

Automated methods:

Detailed instrument settings including instructions for the preparation of the reagents for a variety of automated instruments are available upon request.

Note:

- If higher or lower reactive volumes are required for the method used, the same respective proportions for each reagent concentration, and for the overall reactive volume, must be strictly respected, in order to keep the assay performances.
- Do a sample blank in presence of highly lipemic, icteric or hemolysed plasmas, or if plasma has a "colour" different from the usual one.

QUALITY CONTROL:

Use of quality control plasmas allows validating the calibration curve, as well as the homogeneous reactivity of the BIOPHEN Protein C 5 assay from run to run, and from series to series, when using a same lot of reagents. Various control plasmas are available:

BIOPHEN Normal Control Plasma: (ref A223201).

BIOPHEN Abnormal Control Plasma: (ref A223301).

LIMITATIONS OF THE PROCEDURE:

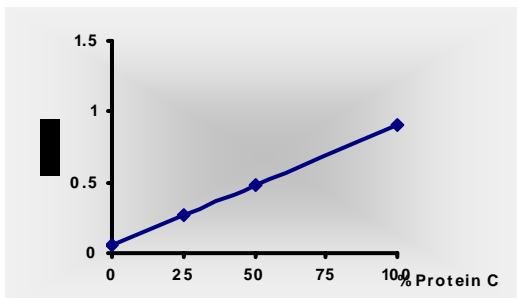
- No significant interference is observed for heparin concentrations < 1 IU/mL, bilirubin concentrations < 0.1 mg/ml, haemoglobin concentrations <1 mg/ml and triglycerides concentrations <1.25mg/ml.
- Aprotinin inhibits Activated Protein C. The "apparent" Protein C activity is decreased in patients treated with aprotinin⁷.
- Presence of anti-human Protein C antibodies in plasma, may inhibit activated Protein C amidolytic activity when performing the assay.
- In order to get the optimal performances of the assay, the procedural instructions must be strictly respected.

RESULTS:

- For the end point method, using a linear graph paper, plot on abscissae the Protein C concentration (%) and on ordinates the corresponding absorbance (A405).
- The Protein C concentration in the tested sample is directly obtained on the calibration curve. Results are expressed as % of a normal plasma pool.
- Using automated methods, the Protein C concentrations are directly calculated by the analyser, respectively to the calibration curve.
- The dynamic range is from 5 to 140 %.

EXAMPLE OF CALIBRATION CURVE:

The calibration curve below is indicated as an example only. Only the calibration curve generated for the series of measures performed must be used.



VALIDATION OF CALIBRATION CURVE:

The calibration curve is acceptable when the concentrations measured for the Control Plasmas are within the acceptance range.

PERFORMANCES AND CHARACTERISTICS:

- 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%.
- 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
Sample 1	98	0.37	9	1.26	12
Sample 2	59	1.17	10	1.97	12
Sample 3	39	0.84	10	1.51	12

EXPECTED VALUES:

By definition, the 100 % Protein C concentration corresponds to the concentration in a normal human citrated plasma pool, obtained by pooling plasmas from healthy males or females aged from 18 to 55 years, and out of any medication. The Protein C concentration in adults is usually between 70 and 140%.

Protein C concentration ≤ 60% indicates the presence of a deficiency, which must be confirmed by another test and / or by testing another plasma sample from the patient.

The Protein C concentration is decreased in neonates.

CLINICAL VARIATIONS:

- A Protein C concentration ≤ 60 % indicates the presence of a deficiency, which must be confirmed by another measurement, or another sample collected from the patient⁶.
- Protein C activity is reduced during dicoumarol therapy, in hepatic diseases, in DIC, or in presence of a congenital or acquired deficiency.

CLINICAL INFORMATIONS:

Protein C deficiencies can be:

- Acquired: they are observed in hepatic diseases, during dicoumarol therapy or in DIC.
- Congenital: they are then associated with recurrent venous thromboses.

Protein C deficiencies can be quantitative (type I) or qualitative (Type II).

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