BIOPHEN Protein C 5

Ref 221205
(R1 R2: 4 x 5 mL)

Chromogenic method for measuring Protein C activity in plasma.

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INTENDED USE:

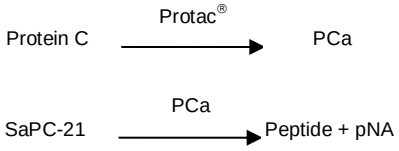
The BIOPHEN Protein C 5 kit is a chromogenic method for *in vitro* quantitative determination of Protein C activity on human citrated plasma[1,2] using manual or automated method.

SUMMARY AND EXPLANATION:

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].
Assay of coagulation Protein C in human plasma may help in 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[3].

PRINCIPLE:

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: Protac®. Highly purified enzyme, extracted from the Agkistrodom C Contortrix snake venom, lyophilized and stabilized, able to specifically activate Protein C. Each vial contains about 1.60U of Protac®. This reagent contains BSA.
4 vials of 5mL.

R2: SaPC-21. Chromogenic substrate, specific for Activated protein C, lyophilized. Each vial contains 8 mg of SaPC-21.
4 vials of 5mL.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially 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.
- 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* diagnostic use.

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

REAGENT PREPARATION AND STABILITY:

The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

R1: Reagent 1: Protac®
Reconstitute the contents of each vial with exactly **5 mL distilled water**, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- **3 months** at 2-8°C.
- **3 days** at room temperature (18-25 °C).
- **Do not freeze.**

R2: Reagent 2: SaPC-21
Reconstitute the contents of each vial with exactly **5 mL distilled water**, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- **3 months** at 2-8°C.
- **3 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
BIOPHEN™ Abnormal Control Plasma	223301
BIOPHEN™ Normal Control Plasma	223201

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 (for the United States, see the CLSI H21-A5 guidelines[8] for further information concerning specimen collection, handling and storage).

- 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[9]:
  - 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 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. 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**.**

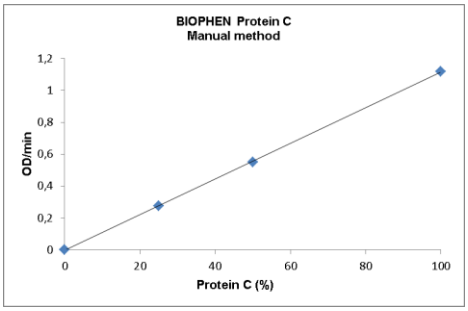
Measure the optical density at 405 nm. Subtract the measured blank value from the absorbance measured for the corresponding test.

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. 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 curve shown below, obtained on manual method 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 concentration, expressed as % 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 % Protein C.
- 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. 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.
- Aprotinin inhibits Activated Protein C. The "apparent" Protein C activity is decreased in patients treated with aprotinin[7].
- Presence of anti-human Protein C antibodies in plasma may inhibit activated Protein C amidolytic activity when performing the assay.
- No significant interference is observed for heparin concentrations <1 IU/mL, bilirubin concentrations <0.1 mg/mL, haemoglobin concentrations <1 mg/mL and intralipids concentrations <1000 mg/dL, by plasma overload test in automated assay.

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%. 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.

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 patient[6].
- Protein C activity is reduced during dicoumarol therapy, in hepatic diseases, in DIC, or in presence of a congenital or acquired deficiency.

Clinical information:

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).

PERFORMANCE:

- 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

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6. Pabinger I.: Clinical relevance of Protein C. Blut 53, 63-75 (1986).
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8. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
9. Woodhams B. *et al*. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.

SYMBOLS:

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