

# PROTEIN C Liquid



Chromogenic assay for measuring Protein C (PC) activity in plasma, with ready to use liquid reagents.

Code MH221211

Packaging R1 3x3 ml + R2 3x3 ml

## INTENDED USE

Meridian Healthcare Protein C (LRT) kit is a chromogenic assay for measuring Protein C activity in human citrated plasma, using a manual or automated method. All the components of this kit are in the liquid, ready to use (LRT = Liquid reagent Technology).

## CLINICAL APPLICATION

Protein C is a plasmatic protein, vitamin K dependent, which inhibits and regulates coagulation through specific cleavage of Factors Va and VIIIa, removing their procoagulant activity. Using the Meridian Healthcare Protein C (LRT) assay, plasmatic Protein C is measured following a specific activation by Protac, a snake venom exact enzyme (Agkistrodom C Contortrix). Then activated protein C (aPC) cleaves the specific substrate SaPC-21 by hydrolysis, releasing para-nitroaniline (pNA), a chromophore group measured at 405nm. There is a direct relationship between colour development and Protein C activity in the tested plasma.

## REAGENT PROVIDED

### R1: Reagent 1: Protac®

Highly purified enzyme, extracted from the Agkistrodom C Contortrix snake venom, stabilised liquid form, able to specifically activate Protein C: 3 vials of 3 ml, containing about 0.32 U/ml of Protac each (ready to use).

### R2: Reagent 2: SaPC-21

Chromogenic substrate, specific for activated Protein C, liquid form: 3 vials of 3 ml, containing about 1.6 mg/ml of SaPC-21 each (ready to use).

## MATERIAL REQUIRED BUT NOT PROVIDED

### Reagents:

- Distilled water, preferentially sterile.
- Acetic Acid (20%) or Citric Acid (2%) (End point method).
- Saline (0.9% NaCl).
- Meridian Healthcare Plasma Calibrator REF MH3700, Protein C reference material (international NIBSC) or internal, calibration plasma with Protein C activity assignment, normal human citrated plasma pool.
- Meridian Healthcare Normal Control Plasma REF MH3110, and Meridian Healthcare Abnormal Control Plasma REF MH3210 (or Normal or Abnormal Plasmas with a known Protein C activity).

### Equipment:

- Spectrophotometer or any analyser capable of chromogenic assays at 405 nm.
- Stop watch.
- Calibrated pipettes.

## STORAGE CONDITIONS

Meridian Healthcare Protein C (LRT) kits must be stored at 2-8°C, in their original packaging box. They are then stable until the expiration date printed on the label.

## PREPARATION AND STABILITY OF REAGENTS

### R1: Reagent 1: Protac

(Clear vial) - Ready to use. Let stand 30 minutes at room temperature (18-25°C), before use. Homogenize before each use.

Stability of Protac®, after opening when kept in its original vial, and avoiding contamination or evaporation:

5 weeks at 2-8°C.

7 days at room temperature (18-25°C).

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

(brown vial) - Ready to use. Let stand 30 minutes at room temperature (18-25°C), before use. Homogenize before each use.

Stability of the substrate, after opening when kept in its original vial, and avoiding contamination or evaporation:

5 weeks at 2-8°C.

7 days at room temperature (18-25°C).

### Caution:

- In order to improve stability, reagents must be closed with their original screw cap following each use (white caps for R1, yellow caps for R2).
- 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. Do not use.
- Stabilisation of the vials, for 30 min. at RT, provides homogeneous reactivity over time.
- To prevent reagent's evaporation, limit the exchange surface by using, for example, a vial reduced neck or a open cap.

## SAMPLES

### Sample collection :

- Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), through a net venipuncture with great care, in order to avoid any activation.

### Centrifugation :

- The centrifugation step is important and is intended to separate the plasma from the platelets. This must be performed within one hour after blood collection using citrate tubes and within 4 hours using plastic tubes. Use a validated method established by your laboratory to obtain platelet poor plasma. For example, 15 minutes at 2000g at room temperature (18-25°C). Use nonactivating plastic tubes and pipettes for handling and storage.

### Plasma Storage :

- 2 hours at room temperature (18-25°C)

## PROCEDURE

Meridian Healthcare Protein C (LRT) kit may used in kinetic methods, automated, but it can also be used manual "for end point" methods. Adaptations for various analysers 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

Calibration is performed with either Meridian Healthcare **Plasma Calibrator (REF MH3700)** or internal or international reference material with a known PC activity ("C") or a normal pooled citrated plasma (made with plasmas from at least 30 normal individuals, healthy males or females, aged between 18 and 55 years, and free of any medication), with the assigned value of 100 % PC, following table below:

| % Protein C | Plasma Calibrator (µl) | Saline (µl) |
|-------------|------------------------|-------------|
| 0           | 0                      | 500         |
| C:4         | 125                    | 375         |
| C:2         | 250                    | 250         |
| C           | 500                    | 0           |

For point at 150%, use the 100% calibrator ("C") 3:4 diluted directly in the reaction (refer below \*). In order to get optimal performance, the calibration curve must be prepared within an hour before running the assay.

## ASSAY PROTOCOL

### Manual Method:

Dilute the samples, the controls and the calibrators 1/2 with saline (0.9% Sodium Chloride).

In a micro plate well, or in a plastic tube pre-incubated 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:                                      |            |           |
| Acetic Acid (20%) or Citric Acid (2%)                                  | 100 µL     | 200 µL    |
| Mix and measure the optical density at 405 nm against the sample blank |            |           |

\* For the 150% PC, introduce 18.75µl of plasma and 6.25µl of Saline in the micro well or 37.5µl of plasma and 12.5µl of Saline in the test tube.

The yellow colour obtained is stable for 2 hours.

The blank is obtained by mixing the reagents in the reverse order from the test i.e.: Acetic Acid (20%) or Citric Acid (2%), SaPC-21 substrate, Protac, diluted plasma.

Measure the Absorbance at 405 nm. Subtract the blank from the absorbance obtained for the assay.

## AUTOMATED METHOD

Method Adaptation for the various analyzers (STA, STA-R, BCT, BCS,...) are available upon request. The assay is then performed kinetically. Refer to each specific adaptation and specific cautions for each instrument.

## QUALITY CONTROL

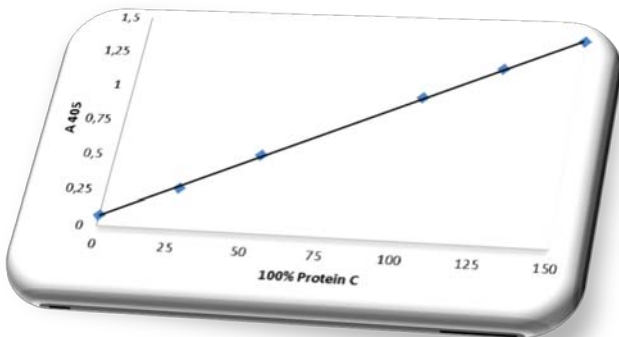
Using Meridian Healthcare **Normal Control Plasma (MH3110)** and Meridian Healthcare **Abnormal Control Plasma (MH3210)**, allows validating the calibration curve, as well as the homogeneous reactivity, when using the same lot of reagents. The calibration curve is acceptable when the measured concentrations for controls are within the assigned range. Various quality control plasmas titrated for PC activity are commercially available, Each laboratory should verify its own target value and acceptance range, in the exact working conditions.

## RESULTS

- For the end-point method, use a **linear** graph paper and plot the calibration curve: Protein C (%) activity on the x-axis and corresponding absorbance on the y-axis.
- Calculate the " $r^2$ " value. Calibration is acceptable if:  $r^2 \geq 0.98$ , and if measured values for controls are in compliance. Using the manual method (test tube), the expected absorbance values range is 0 for the 0% PC, to about 0.90 ( $0.90 \pm 0.15$ ) for the 100% PC. For information, expected absorbance using the microplate method, is lower than using the test tube method. Absorbance values can differ according to the instrument application used.
- The PC concentration in the tested sample is directly obtained on the calibration curve (concentration corresponding to the measured A405). Results are expressed as % PC.
- The dynamic range is from 0 to 100% and can be up to 150 % PC. When the assay dilution is **1:2**, the PC concentration is directly read on the calibration curve. When predilutions are used, multiply the measured PC concentration by the predilution factor in order to get the concentration in the tested specimen.
- Using automated methods, the %PC are directly calculated by the analyser, using the calibration curve, and the sample dilution used.
- Alternatively, statistics software can be used to establish the dose response calibration curve. A linear relationship is obtained between % PC and Absorbance.

## EXAMPLE OF CALIBRATION CURVE

The calibration curve below is provided as an example only. Only the calibration curve generated for measured the series must be used.



## PERFORMANCES AND CHARACTERISTICS

- **Dynamic range: 0 to 150 % PC** (ie 0 to 1.5 IU/ml PC).
- The detection limit is calculated by measuring on the curve the "observed" Protein C level, which correspond the average absorbance observed for a Protein C deficient sample plus 3 standard deviations (SD). This detection threshold is  $\leq 5\%$ .
- Reproducibility: expected CV  $\leq 2\%$ .

## LIMITATION OF PROCEDURE

- No significant interference is observed for heparin concentrations up to 1IU/ml, bilirubin concentrations  $<0.28$  mg/ml, haemoglobin concentrations  $<1.5$  mg/ml and triglycerides concentrations  $<3$ mg/ml.
- Aprotinin inhibits Activated Protein C. The "observed" 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 during the assay.
- In order to get the optimal assay performances, the working instructions must be carefully observed. Each laboratory should verify performances in its exact working conditions.

## EXPETED VALUES

By definition, 100 % corresponds to the Protein C 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 healthy adults is between 70 and 140%. The Protein C concentration is decreased in neonates due to hepatic immaturity. It is later independent of age and sex.

## CLINICAL VARIATION

A Protein C concentration  $\leq 60\%$  indicates the presence of a deficiency, which must be confirmed by another measurement, or another sample collected from the patient .

Protein C activity is reduced during Anti vitamin K (AVK) 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 AVK therapy or in DIC.
- Congenital: they are then associated to recurrent venous thrombosis.

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

## BIBLIOGRAFY

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## SIMBOLOGY

| LEGENDA DEI SIMBOLI |  |  |                 |  |                 |
|---------------------|--|--|-----------------|--|-----------------|
|                     | Attenzione, consultare le istruzioni per l'uso       |  | Test per kit    |  | Produttore      |
|                     | Unicamente per diagnosi in vitro                     |  | Scadenza        |  | Monouso         |
|                     | Conservare ad una temperatura compresa tra 2° e 30°C |  | Numero di lotto |  | Codice prodotto |

## PRODUTTORE

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