DIAGON
Solutions beyond imagination

INSTRUCTION FOR USE

Dia-PROTEIN C

Cromogenic reagent

CE

Cat.No.: 07108 Dia-PROTEIN C 4x2 ml

Intended use

The Diagon Ltd Protein C (Chromogenic) assay is intended for the quantitative determination of protein C in human plasma using a chromogenic assay method. Protein C is a vitamin K dependent protein which plays an important role in the regulation of anticoagulant mechanisms. It can inhibit coagulation by inactivating factors Va[1] and Vllla[2] or, when activated, can stimulate fibrinolysis[3]. Protein C circulates as a zymogen, and is converted to an active serine protease by the action of thrombin in the presence of thrombomodulin. Both hereditary and acquired Protein C deficiencies have been shown to be a risk factor for development of venous thrombosis. Protein C in plasma is activated by a specific fraction from the *Agkistrodon contortrix* snake venom. The amount of activated protein C (APC) is determined by monitoring the rate of hydrolysis of a protein C specific chromogenic substrate. The release of pNA is measured at 405 nm and is proportional to the protein C level.

Warnings

The reagents contained in this kit are for *in vitro* diagnostic use only under professional's leadership.

Precaution

The reagents contained in this kit are for *in vitro* diagnostic use only - **DO NOT INGEST!** Wear gloves when handling all kit components.

Waste Material Treatment

Refer to the product safety data sheets for risk and safety phrases and disposal.

Materials provided

Component	Content	Description	Preparation
Protein C Substrate	4 x 2 mL	Each vial contains 2.75 µmol Iyophilised pyro-Glu-Pro-Arg-pNA.HCl.	Reconstitute each vial with 2 mL of Protein C Diluent. If cloudy, warm at $^{+}$37°C for a few minutes.
Protein C Activator	4 x 2 mL	Each vial contains 0.8 units of activator from snake venom (Protac®).	Reconstitute each vial with 2 mL deionised water.
Protein C Diluent	2 x 5 mL	Each vial contains this buffer with sodium azide as a preservative.	Ready for use.
Each kit contains Instructions For Use.			

Materials required but not provided

Cat.No.: 09605 Dia-Cal Spec

Cat.No.: 09305 Dia-Cont Spec N

Cat.No.: 09405 Dia-Cont Spec P

Storage and stability

Unopened vials are stable until the given expiry date when stored under the related conditions.

Protein C Substrate	Reconstituted reagent is stable for 1 week at $^{+}$2–$^{+}$8°C or one month at -20°C.
Protein C Activator	Reconstituted reagent is stable 1 week at $^{+}$2–$^{+}$8°C or one month at -20°C.
Protein C Diluent	Store at $^{+}$2–$^{+}$8°C.

Sample Collection and Preparation

Plastic or siliconised glass should be used throughout. Blood (9 parts) should be collected into 3.2% or 3.8% sodium citrate anticoagulant (1 part). Separate plasma after centrifugation at 1500 x g for 15 minutes. Plasma should be kept at $^{+}2$ – $^{+}8$°C or $^{+}18$ – $^{+}24$°C. Testing should be completed within 4 hours of sample collection, or plasma can be stored frozen at -20°C for 2 weeks or -70°C for 6 months. Thaw quickly at $^{+}37$°C prior to testing. Do not keep at $^{+}37$°C for more than 5 minutes[4]. Erroneous results may be caused by contamination with tissue fluids or stasis. Avoid agitation, air bubbles or foaming. For the effects of commonly administered drugs, refer to Young, *et al*[5].

Procedure

Prepare plasma standards and patients' plasma samples as follows:

A. Manual Method

Important: Use only Dia-Saline Solution for dilutions.

Standard %	Plasma	Dia-Saline
100%	100 µL Calibration Plasma	+ 300 µL saline
50%	50 µL Calibration Plasma	+ 350 µL saline
0%		400 µL saline only
Patient	100 µL plasma	+ 300 µL saline

Manual Method on Coag 4D semi automatic coagulometer

1) Dilute 1 part patient plasma or reconstituted control with 3 parts Saline Solution and mix them.
2) Take to the cuvette 60 µL diluted calibrator, control or patient sample, and incubate at $^{+}37$°C for 30 secundum. **In case of software versions before 2.06 for Coag4D and 2.02 for Coag2D you have to control the indicated incubation time by stopwatch!**
3) In the case of software versions 2.06 (for Coag4D) and 2.02 (for Coag2D) this step has already been built in the software.
4) Add 60 µL Protein C Activator and incubate the mix at $^{+}37$°C for 5 minutes.
5) Add 60 µL Protein C Substrate reagent.
6) Measure the absorbance change with 0.1 sec accuracy.
7) Plot the Protein C activity (X-axis) versus the absorbance change (Y-axis) on linear graph paper.
8) Interpolate the patient and control values from the straight calibration line.

B. End Point Method Assay

1. To a glass or plastic test tube:
   a. Add 100 µL standard or patients' plasma dilution.
   b. Incubate at $^{+}37$°C for 2 minutes.
   c. Add 200 µL Protein C Activator and mix.
   d. Incubate at $^{+}37$°C for 5 minutes.
   e. Add 200 µL Protein C Substrate and mix.
   f. Incubate at $^{+}37$°C for 10 minutes
   g. Add 200 µL acetic acid and mix.
   h. * Add 200 µL water (optional).

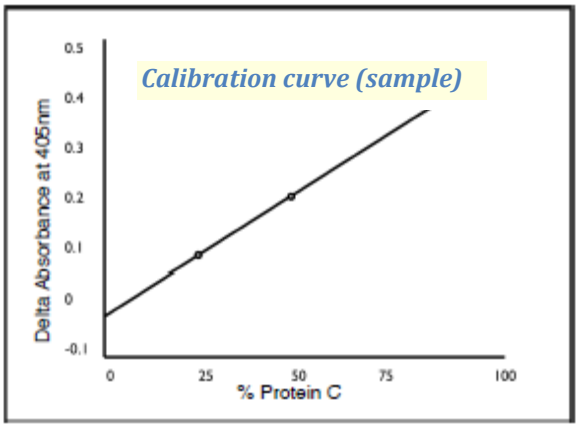
* Some spectrophotometers require a minimum of 1 mL volume in the cuvette.

2. Read absorbance at 405 nm in a 1 cm semi-micro cuvette against a blank prepared with deionised water. As many as ten determinations can be performed simultaneously with the same stop watch by staggering pipetting steps at five second intervals.

Calibration Curve

Plot the absorbance obtained for each of the protein C standards against protein C % on linear graph paper. The protein C concentration in patient's plasma specimens can be determined by interpolation from the calibration curve.

If a commercial protein C standard is used, the protein C concentration in the patient's specimen should be adjusted for the protein C concentration in the standard. The calibration curve shown below is an example only. A calibration curve must be obtained each time the assay is performed



X axis: % Protein C
Y axis: Absorbation change on 405nm

B. Automated Kinetic Method

Contact Diagon Ltd for instrument-specific application guides. Example table:

Interpretation of results

Protein C deficiencies, either congenital or acquired, may lead to serious thrombotic events such as thrombophlebitis, deep vein thrombosis, or pulmonary embolism. Approximately 2-8% of all patients with venous thrombosis under the age of 40-45 years have Protein C deficiencies.

Patients with hereditary deficiencies generally present with venous thrombosis in young adulthood. The first episode is usually spontaneous and associated with trauma or stress to the haemostasis mechanism. The prevalence of Protein C deficiencies is one case per 300 people or approximately 0.33%.

Acquired Deficiencies
Decreased levels of protein C are observed in the following cases:

1. Hepatic disorders: hepatitis, cirrhosis.
2. DIC.
3. Oral anticoagulant therapies, in which cases the interpretation of test results is difficult if the patients have had a history of thromboses and are receiving oral anticoagulant treatments.

Limitations

Icteric, haemolysed or hyperlipemic specimens interfere with absorbance readings, thus requiring the use of plasma blanks for accurate results. Plasma blanks are also needed on patients when contact factor activation is suspected, such as DIC patients, or from individuals on oral contraceptives where cold activation may occur. Blanks are obtained by substituting saline for the Protein C Activator in the test reaction. Subtract the sample blank activity from the sample test activity. The naturally-occurring activator of protein C is thrombin in the presence of thrombomodulin. The possible existence of clinical protein C abnormalities not detectable by reactivity with the snake venom activator or with the chromogenic substrate used in this kit should not be precluded. To assure accurate, reproducible results, use accurate pipetting devices and observe recommended procedures with particular emphasis on incubation times and incubation temperature.

Quality control

Each laboratory should establish a quality control program. Normal and abnormal control plasmas should be tested prior to each batch of patient samples, to ensure satisfactory instrument and operator performance. If controls do not perform as expected, patient results should be considered invalid. Diagon Ltd supply the following controls available for use with this product:

Cat.No.: 09305 Dia-Cont Spec N

Cat.No.: 09405 Dia-Cont Spec P

Reference values

Reference values can vary between laboratories depending on the techniques and systems in use. For this reason each laboratory should establish its own normal range. Protein C activity values are usually expressed in relative percentages compared to a pooled normal plasma standard. Bertina *et al*[6] reported a range of 65-145% in healthy individuals with diminished levels found following anticoagulant therapy. There is apparently no difference in protein C between healthy males and females[7].
The following reference values have been determined by Diagon Ltd using an optical coagulation instrument, using 17 plasmas of presumed healthy men and women.

Protein C activity %	**SD**	**Range**
105%	26.6%	61.2-162.9%

Performance characteristics

Diagon Ltd or their representatives have determined the following performance characteristics as a guideline.

Each laboratory should establish it's own performance data.

Specificity

In studies where recovery of protein C plasma was determined after addition of various amounts of protein C to protein C deficient plasma, the expected recovery was obtained.

Reproducibility

	Intra-assay precision		**Inter-assay precision**		
n	***Protein C***	***CV (%)***	***n***	***Protein C***	***CV (%)***
10	68.5	2.5	18	45.3	2.6

References

1. Walker FJ (1981) Regulation of activated protein C by protein S. The role of phospholipid in factor V inactivation, *J BiolChem*, **256:** 11128-31
2. Taylor FB, Lockhart MS (1985) A new function for activated protein C: activated protein C prevents inhibition of plasminogen activators by releasate from mononuclear leukocytes—platelet suspensions stimulated by phorboldiester, *Thromb Res*,**37(1):** 155-64
3. Fulcher CA, Gardiner JE, Griffin JH, Zimmerman TS (1984) Proteolyticin activation of human factor VIII procoagulant protein by activated human protein C and its analogy with factor V,**63(2):** 486-9
4. Clinical and Laboratory Standards Institute (2008) Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Haemostasis Assays: Approved Guideline, 5th edn. CLSI: H21-A5
5. Young DS *et al.* Effects of Drugs on Clinical Laboratory Tests, 3rd ed., AACC Press, Washington, D.C.,1990
6. Bertina RM, Broekmans AW, Krommenhoek-Van Es C, Van Wijngaarden A (1984) The Use of a Functional and Immunologic Assay for Plasma Protein C in the Study of the Heterogeneity of Congenital Protein C Deficiency, *ThrombHaemostas*, **51:** 1-5
7. Pabinger-Fasching I, Bertina RM, Lechner K, Niesser H, Korninger C (1983) Protein C Deficiency in Two Austrian Families, *ThromHaemostas*,**50:** 810-3

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Symbols			
IVD	In vitro diagnostics devices		Check in user manual
	Biohazard		Temperature range
	Manufacturer		Expiry date
LOT	LOT number	CE	CE conformity sign
REF	Catalogue number		