DIAGON
Solutions beyond imagination

INSTRUCTION FOR USE

Dia-PROTEIN S

Clotting reagent

CE

Cat. No.: 07204 Dia-Protein S 4x1 ml

Intended Use

Determination of functional Protein S levels in human plasma by clotting assay. Protein S is a vitamin K-dependent protein synthesized in the liver, vascular endothelium, and megakaryocytes, which plays an important physiological role in the Protein C Anticoagulant System[1,2]. This anticoagulant system is one of the major regulators of haemostasis by inhibiting clot formation and by promoting fibrinolysis. Protein S functions as a cofactor for activated Protein C to facilitate the degradation of clotting factors Va and VIIIa, down-regulating clot formation[3].

In normal plasma approximately 40% of Protein S circulates as a free molecule, while 60% is complexed with C4b, a plasma protein of the classical complement pathway. Only Free Protein S is functionally active and able to bind to activated Protein C, while the complexed form of Protein S is not[4]. The Dia-PROTEIN S (Clot) assay determines the amount of functional Protein S in plasma.

In this assay, dilutions of normal plasma are mixed with Protein S depleted plasma. The mixed plasma is then activated by reagents which contain activated protein C, other human plasma proteins and phospholipids. After activation, clotting is initiated by the addition of calcium chloride. Human aPC and other plasma proteins are added at such quantities, so as to normalise any deficiencies in the patient sample, preventing false positive or negative results for Protein S functionality. The prolongation of the clotting time is thus directly related to the concentration of the Protein S in the patient plasma.

Warnings

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

Precaution

Potentially dangerous material – **DO NOT INGEST!**

Wear gloves when handling all kit components. Plasma products have been screened and found negative for the presence of Hepatitis B Antigen (HbsAg) HIV 1 and 2 antibody and HCV antibody; however they should be handled with the same precautions as a human plasma sample. All immunodepleted plasmas are HCV negative

Waste Material Treatment

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

Materials provided

Component	Content	Description	Preparation
Protein S Activator	4 x 1 mL	Activated Protein C, Polybrene® and bulking agents.	Reconstitute with 1 mL purified water. Allow to stand for 20 minutes and mix well before use, do not shake.
Protein S Deficient Plasma	4 x 1 mL	Immunodepleted Protein S deficient human plasma.	Reconstitute with 1 mL purified water. Allow to stand for 20 minutes and mix well before use, do not shake.
Protein S Substrate	4 x 1 mL	Human plasma proteins and stabilisers.	Reconstitute with 1 mL of Substrate Diluent. Allow to stand for 20 minutes, and mix well before use, do not shake.
Substrate Diluent	2 x 2 mL	Ellagic Acid, phosholipids, buffers, stabilisers and preservatives (including 0.2% phenol).	The reagent is ready for use as packaged. Gently invert the vials several times until a homogenous suspension is obtained.
Calcium Chloride Solution 0.025M	2 x 5 mL	0.025 M Calcium Chloride.	The reagent is ready for use as packaged.
Saline Solution: 0.9%	2 x 25 mL	0.9% sodium chloride, and 0.09% sodium azide as a preservative.	The reagent is ready for use as packaged.

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 S Activator	Reconstituted vials are stable for up to 1 week at +2–+8°C, or 8 hours at room temperature. **DO NOT FREEZE!**
Protein S Deficient Plasma	Reconstituted vials are stable for 8 hours at +2–+8°C, 4 hours at room temperature and 1 month at -20°C if thawed only once. **DO NOT FREEZE!**
Protein S Substrate	Reconstituted vials are stable for up to 8 hours at +2–+8°C, or 3.5 hours at room temperature. **DO NOT FREEZE!**
Substrate Diluent	Opened vial should be stable until the given expiry date. **DO NOT FREEZE!**
Calcium Chloride Solution 0.025M	Opened vial should be stable until the given expiry date. **DO NOT FREEZE!**
Saline Solution: 0.9%	Opened vial should be stable until the given expiry date. **DO NOT FREEZE!**

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[5].

Procedure

It is recommended that tests be performed in duplicate.

Note: A new standard curve must be constructed each time the assay is performed. Ensure that a sufficient quantity of each reagent is reconstituted to complete all tests. Pool vials if necessary.

A. Manual method

1. Reconstitute the required vials of reagent. Place the Dia-CaCl2 Calcium Chloride solution into a +37°C incubator.
2. Prepare a stock standard by mixing 200 µL of reference plasma with 1800 µL of Saline Solution.
3. Use the stock standard immediately to make the following standard dilutions:

Dilution	Volume of standard (µL)	Volume of saline (µL)	Activity %
1	400	0	100
2	300	100	75
3	200	200	50
4	100	300	25
5	0	400	0

4. Prepare patient and control dilutions by adding 50 µL of plasma to 450 µL of Saline Solution. Keep all standard, patient and control dilutions on ice and test within 30 minutes.
5. To a coagulation cuvette add 30 µL Protein S deficient plasma.
6. Add 30 µL standard, control or patient dilution.
7. Add 30 µL Protein S Activator reagent.
8. Add 30 µL of Protein S Substrate reagent.
9. Incubate exactly for 4 minutes at +37°C.
10. Add 30 µL Calcium Chloride solution and start timer.
11. Note clotting time to the nearest 0.1 seconds.
12. Plot the Protein S activity (X-axis) versus clot time (Y-axis) on linear graph paper.
13. Interpolate patient and control values from the straight calibration line.

B. Automated method

Refer to the appropriate Instrument Operator Manual for detailed instructions or contact Diagon Ltd. for instrument-specific application guides.

Interpretation of results

Protein S deficiency, either congenital or acquired, may lead to serious thrombotic events such as thrombophlebitis, deep vein thrombosis, or pulmonary embolism. The prevalence of Protein S deficiency has been estimated to be less, than 1 case per 300 in the general population. Two-thirds of patients with a congenital deficiency of Protein S (levels less than 50% of normal) may present with venous thrombosis in young adulthood[6,7]. In young patients (<35 years) with a history of thrombosis, the prevalence may be as high as 15% to 18%[8]. Acquired Protein S deficiency may be seen during pregnancy, oral contraceptive or oral anticoagulant therapy, liver disease, diabetes mellitus, postoperative complications, septicemia and various inflammatory syndromes[9].

A decreased Protein S activity in plasma may be the result of low concentrations or abnormal function of the Protein S molecule. A decrease in Protein S is associated with increased incidence of thromboembolism[10]. A decrease in Protein S activity does not necessarily indicate a decrease in concentration. The laboratory diagnosis of Protein S deficiency may require both qualitative (functional) and quantitative (antigen level) determinations[11].

Limitations

1. >150% PS. Patients with high levels of Protein S should be tested at multiple dilutions to obtain accurate results. Corrected Protein S levels from at least 2 dilutions must agree.
2. Heparin/Warfarin Interference. Heparin at levels >1 U/mL may interfere by prolonging clot times and hence increasing apparent Protein S values. Warfarinisation may affect Protein S results.
3. LA/APA. Lupus antibody positive samples with prolonged aPTT times may give false high Protein S values and should be confirmed by free antigen assay.
4. Factor V Leiden. Borderline or low functional Protein S levels on patients with Factor V Leiden should be confirmed by free Protein S antigen assay.
5. Thrombin Inhibitor Interference. Thrombin inhibitors (hirudin, bivalirudin, etc.) in the patient sample could lead to an over-estimation of Protein S.

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, the patient specimen measurement should not be started.

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. The expected normal range for Protein S is gender specific.

Males	63,5 – 167,9%
Females	56,7 – 147,2%

Performance characteristics

The following performance characteristics have been determined by Diagon Ltd. using an optical coagulation instrument. Each laboratory should establish its own performance data.

Reproducibility

		Intra-assay precision		Inter-assay precision	
Sample	***n***	***Protein S Level (%)***	***CV (%)***	***Protein S Level (%)***	***CV (%)***
Speciality Assayed Control P	10	68.6	1.55	68.9	3.61
Speciality Assayed Control N	10	156.8	2.57	151.9	4.90

References

1. DiScipio RG, Davie, EW (1979) Characterisation of Protein S; a gamma-carboxyglutamicacid containing protein from bovine and human plasma, *Biochemistry*,**18:**899-904
2. Dahlback B (1984) Purification of human vitamin K-dependent Protein S and its limited proteolysisbythrombin, *Biochem J*,**209:**837-846
3. Walker FJ (1984) Protein S and the Regulation of Activated Protein C, *Semin. Thromb. Hemost*,**10:**131-138
4. Dahlback B (1991) Protein S and C4b-Binding Protein: Components involved in the regulation of the Protein C Anticoagulant System, *Thromb. Haemost*,**66:**49-61
5. 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
6. Comp PC, Nixon RR, *et al.* Familial Protein S deficiency is associated with recurrent thrombosis, *J. Clin. Invest*,**74:**2082-2088
7. Walker FJ (1992) Protein S and Thrombotic Disease, *PSEBM*,**200:**285-295
8. Gladson CL *et al.* (1988) The frequency of type I heterozygous Protein S and Protein C deficiencyin 141 unrelated young patients with venous thrombosis, *Thromb. Haemost*,**59:**18-22
9. Alving BM, Comp PC (1992) Recent advances in understanding clotting and evaluating patients with recurrent thrombosis, *Am. J. Obstet. Gynecol*, **167:**1184-1191
10. Bertina RM (1985) Hereditary Protein S deficiency, *Haemostasis*,**15:**241-245
11. Comp PC (1990) Laboratory evaluation of Protein S status, *Semin. Thromb. Haemost*,**15:**177-181

DIAGON LTD.
Baross u. 48-52, 1047 Budapest, Hungary
Tel.: +36 1 3696500
Fax.: +36 1 3696301
Web: www.diagon.com
E-mail: diagon@diagon.com

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		