BioMedica Diagnostics Inc.
P.O. Box 1030, 94 Wentworth Road
Windsor, NS, Canada B0N 2T0
1.902.798.5105
www.biomedicadiagnostics.com

ACTICLOT® Protein S

REF 843L-HS

IVD

CE

Σ 80

2°C 8°C

EC REP **Obelis s.a**
Bd. Général Wahis 53, 1030 Brussels, BELGIUM

INTENDED USE

ACTICLOT® Protein S is a functional clotting assay intended for the quantitative determination of Protein S activity in human plasma. The assay is for *in vitro* diagnostic use.

EXPLANATION OF THE TEST

DiScipio and colleagues first identified human Protein S (S for Seattle) in 1977.[1] Human Protein S is a single chain glycoprotein of 635 amino acids (69,000 kD) that circulates in plasma at a concentration of about 320 nM. The active Protein S gene has 15 exons and spans more than 80 kilobases on Chromosome 3.[2] Protein S is a vitamin K-dependant protein primarily synthesized in the liver but also in the endothelium and possibly in megakaryocytes. It is possible that all three sources of Protein S play a significant role in regulating haemostasis.

Protein S did not appear to be the zymogen precursor of a serine proteinase and no function was known until 1980 when Walker reported that the bovine Protein S functions as a cofactor for activated Protein C in the inactivation of activated factor V.[3] The biologic importance of the regulation of coagulation by the pathway involving Protein C, Protein S and thrombomodulin has been substantiated by clinical studies. An association between thrombosis and partial deficiencies of Protein S has been documented in certain families[4,5,6]. Protein S has been also found on the surface of endothelial cells and platelets[7]. Approximately 60% of Protein S in human plasma is tightly bound to C4b-binding protein, therefore, plasma levels of Protein S may not completely reflect its bioavailability[8]. This complex has no anticoagulant cofactor activity. It has been suggested that Protein S may have a role in the complement system by mediating the association of C4b-binding protein (C4bBP) with cell surfaces that have been damaged or activated to expose negatively charged phospholipids[9]. This may represent another important regulatory mechanism for Protein S.

PRINCIPLE OF THE METHOD

The ACTICLOT Protein S assay is a clotting-based plasma assay. In the assay, dilutions of patient plasma are mixed with Protein S depleted plasma. An activator

reagent containing factor Xa, activated Protein C and phospholipids is added to the mixture. Following a 5-minute incubation period, calcium chloride is added to initiate clot formation. Under these conditions, the prolongation of the clotting time is directly proportional to the concentration of Protein S in the patient plasma.

REAGENTS

The kit contains reagents sufficient to perform 80 tests using an automated coagulation analyser, 40 tests if a manual end-point method is used.

R1 ACTICLOT Activator Reagent: 4 vials, 1.0 mL (lyophilised)

R2 Protein S Deficient Plasma: 6 vials, 1.0 mL (lyophilised)

R3 Sample Dilution Buffer: 2 vials, 2.5 mL 10X concentrate

WARNING

This product contains human source material that has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using registered methods. As no known test method can provide complete assurance that products derived from human specimens will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, this product should be handled as recommended for any potentially infectious human specimen.

This product contains animal source material. As no known test method can provide complete assurance that products derived from animal specimens will not transmit blood-borne pathogens, this reagent should be handled as recommended for any potentially infectious specimen.

REAGENT PREPARATION AND STORAGE

Lyophilized and concentrated liquid reagents and are stable until the expiration date indicated on their vial labels when stored at 2° - 8°C.

A. ACTICLOT Activator Reagent (R1)

Reconstitute with 1 mL Type I deionised or distilled water. Let stand at room temperature (18° - 25°C) for 20 minutes to allow for complete dissolution. Then keep at 2° - 8°C and use within 4 hours. Reconstituted Activator Reagent is stable for:

	30 days
	-20°C

B. Protein S Deficient Plasma (R2)

Reconstitute with 1 mL Type I deionised or distilled water. Mix gently and let stand at room temperature (18° - 25°C) for 20 minutes to allow for complete dissolution. Then keep at 2° - 8°C and use within 4 hours. Reconstituted Deficient Plasma is stable for:

	30 days
	-20°C

C. Sample Dilution Buffer (R3)

Dilute contents of one vial to 25 mL with Type I deionised or distilled water and mix. Working strength Dilution Buffer is stable for:

	30 days
	2° - 8°C

SPECIMEN COLLECTION AND PREPARATION

See “Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition", CLSI Document H21-A5, Vol. 28, No. 5, 2008[10]. Plasma collection should be performed as follows:

1. Collect 9 volumes of blood in 1 volume of 0.1 M trisodium citrate.

2. Centrifuge the blood sample at 2,500 x g for 15 minutes.

3. Plasma should be stored at 2° to 8°C and assayed within 2 hours. Alternatively, plasma may be stored for:

	1 month
	-20°C

4. Frozen plasma may be thawed once at 37°C, 30 minutes before use.

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

Type 1 deionised water or distilled water
Special Coagulation Calibrator, REF C.BMD.SCC030-01ML-A
Special Coagulation Control Normal, REF C.BMD.SCCN180-01ML-A
Special Coagulation Control Abnormal, REF C.BMD.SCCA180-01ML-A
0.025 M Calcium Chloride solution
Automated coagulation analyser or mechanical clot timer
25 mL graduated cylinder
variable volume pipettor (100-1000 µL)
Incubator or water bath at 37°C

Assay Calibration

The Special Coagulation Calibrator, REF C.BMD.SCC030-01ML-A, should be used for preparation of the Protein S calibration standards. Prepare Protein S calibration standards, control and patient plasma samples as follows. Use the standards and samples immediately after preparation.

Protein S Standard*	Volume of Special Coagulation Calibrator	Volume of Dilution Buffer
100%	100 µL	900 µL
50%	500 µL of 100% Standard	500 µL
12.5%	250 µL of 50% Standard	250 µL
0%	0 µL	1000 µL
Patient Sample/Control	Patient Sample/Control	Volume of Dilution Buffer
100%	50 µL	450 µL

* The actual value of the Protein S Standards will depend on the lot specific value of the Special Coagulation Calibrator.

Assay Procedure

ACTICLOT Protein S may be performed manually, or by using semi-automated or automated coagulation analysers.

Automated Coagulation Analyser Method

BioMedica Diagnostics offers instrument applications for performing ACTICLOT Protein S on several coagulation analysers. These instrument applications may contain platform specific programming and performance data which differ from that provided in this Instructions for Use. In these cases, the information contained in the instrument application supersedes the information in this Instruction for Use. Please consult the specific manufacturer's instrument manual for complete operating instructions.

Manual End-point Method

Transfer ACTICLOT Activator and 25 mM calcium chloride solution to 37˚C reagent wells. Incubate for at least 2 minutes.

1. Add 0.1 mL of Protein S Deficient Plasma + 0.1 mL standard dilution or patient sample to a coagulation cuvette.

2. Incubate at 37°C for 2 minutes.

3. Add 0.1 mL of ACTICLOT Activator.

4. Incubate at 37°C for 5 minutes.

5. Add 0.1 mL of 0.025 M Calcium Chloride solution.

6. Start clot timer and note the clotting time.

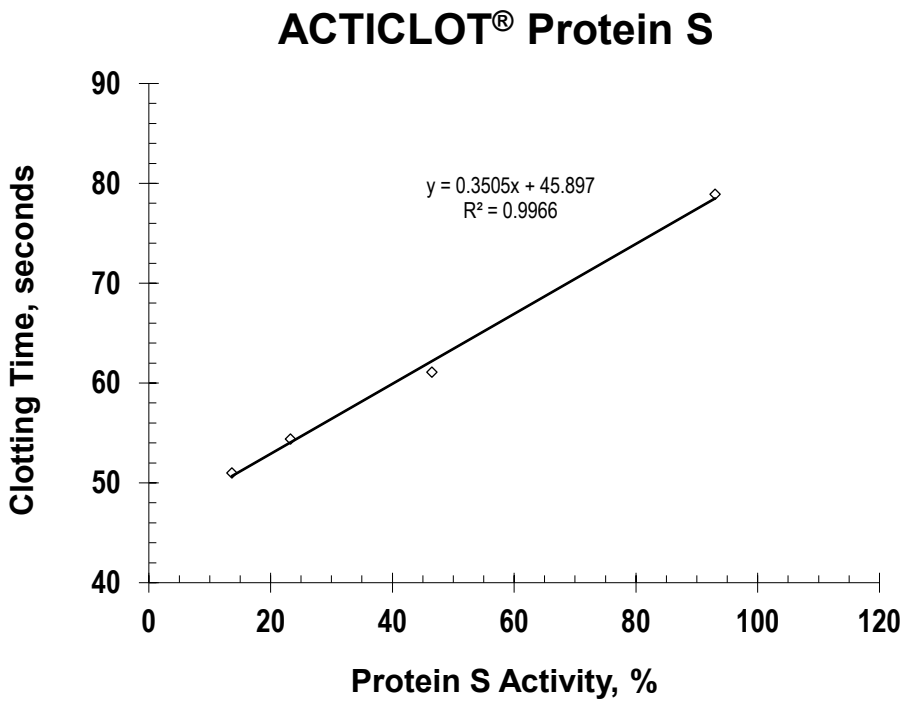
7. Obtain duplicate determinations for each plasma dilution.

RESULTS

Representative Standard Curve

A standard curve is constructed by plotting the mean clotting time for each Protein S standard versus its corresponding activity in percent. A standard curve should be generated each time the assay is performed. Draw the line of best fit between the points, typically a linear equation, for data analysis.

The following standard curve is for demonstration purposes only.



CALCULATION OF RESULTS

Determine the % Protein S in the test sample by interpolating from the standard curve. In the case of patients with lupus anticoagulants or abnormally high Protein S activity, where multiple patient dilutions were assayed, correct the Protein S level for the dilution. Corrected Protein S levels from at least two dilutions must agree.

QUALITY CONTROL

Control plasmas should be included in the run whenever freshly reconstituted reagents are used. The Special Coagulation Control Normal, REF C.BMD.SCCN180-01ML-A, and the Special Coagulation Control Abnormal, REF C.BMD.SCCA180-01ML-A, may be used. The levels obtained for these control plasmas should fall within the specified ranges. If these controls plasmas fail to yield Protein S levels within the range specified, the run should be repeated. Contact BioMedica Diagnostics if repeated assaying of these control plasmas fails to yield Protein S levels within acceptable limits.

LIMITATIONS OF THE PROCEDURE

The presence of heparin greater than 1.2 U/mL in the patient sample, or the presence of lupus anticoagulants in the sample, may affect the assay results by prolonging the clotting time, giving an artificially high Protein S value. These samples should be tested at multiple dilutions to correct this effect.[11]

Functional assays for Protein S can be influenced by the presence of factor V Leiden mutation in the patient, as this condition inhibits the Protein S enhanced inactivation of factor Va by activated Protein C.

This test should not be used as the sole means of diagnosis. Standard coagulation testing regimen should be followed.

EXPECTED VALUES

The value for Protein S is usually expressed as a relative percent compared to the value of a standard or pooled normal plasma. The expected normal range for Protein S is 55-160%.[12] However, each laboratory should determine its own normal range.[13] Males have 10%-15% more plasma Protein S than females, and the concentration increases somewhat with age in both genders.[9] A decrease in Protein S is associated with an increased incidence of thromboembolism.[14] A decrease in Protein S activity does not necessarily indicate a decrease in Protein S plasma concentration. As Protein S (total) is present in plasma as free protein and as protein bound to C4bBP, only the free Protein S acts as a cofactor for activated Protein C. When the Protein S activity is decreased, it is important to establish the plasma levels of both free Protein S and C4bBP bound Protein S. Three types of congenital Protein S deficiency have been described, as summarized in Table 1.[15]

Table 1

DEFICIENCY TYPE	TOTAL PROTEIN S	FREE PROTEIN S	PROTEIN S ACTIVITY
I	∇[b]	∇	∇
II	⇔[a]	⇔	∇
III	⇔	∇	∇

a: ⇔ = is normal

b: ∇ = is at a reduced level

Levels of Protein S may also be decreased in liver disease and during anticoagulant therapy. In addition, Protein S activity and free Protein S antigen may be reduced in inflammatory disease, in which the levels of C4bBP are elevated.

INTERFERING SUBSTANCES

The following substances at the indicated levels may interfere with ACTICLOT Protein S.

Substance	Concentration
Hemoglobin	> 500 mg/dL
Bilirubin	> 21 mg/dL
Triglycerides	> 900 mg/dL

PERFORMANCE CHARACTERISTICS

The end-user should establish product performance characteristics for the specific method or instrumentation used. Studies using an automated analyser with photo-optical detection produced the results shown below[11].

Accuracy

A study performed using another commercially available Protein S kit gave a correlation coefficient of 0.868 for 78 samples tested, with a regression equation of y = 0.913x + 17.2.

Precision

The following coefficients of variation (CV's) were observed:

Protein S Level	Intra-Assay CV	Inter-Assay CV
Normal Plasma (87%)	3.0%	5.9%
Abnormal Plasma (42%)	2.3%	5.1%

Sensitivity

ACTICLOT Protein S is designed to give a linear standard curve for Protein S levels between 0 and 100% when plotted as directed.

REFERENCES

1. Discipio, R. G., *et al*. A comparison of human Prothrombin, factor IX (Christmas Factor) factor X (Stuart Factor) and Protein S. *Biochemistry* 1977, **16**: 698-706.
2. Schmidel, D. K. Organization of the human Protein S genes. *Biochemistry* 1990, **16**: 7845-7852.
3. Walker, F. J. Regulation of activated Protein C by a new protein. A possible function for bovine Protein S. *Journal of Biological Chemistry* 1980, **255:** 5521-5524.
4. Comp, P. C., *et al*. Familial Protein S deficiency is associated with recurrent thrombosis. *Journal of Clinical Investigations* 1984, **74**: 2082-2088.
5. Broekmans, A. W., *et al*. Hereditary Protein S deficiency and venous thromboembolism. A study in three Dutch families. *Thrombosis and Haemostasis* 1985, **53**: 273-277.
6. Kamiya, T., *et al*. Inherited deficiency of Protein S in a Japanese family with recurrent venous thromboembolism. A study of three generations. *Blood* 1986, **67**: 406-410.

7. Sschwarz, H. P., *et al*. Identification and quantification of Protein S in human platelets. *Blood* 1985, **66**: 1452-1455.
8. Dahlbäck, Björn. Interaction between vitamin K-dependant Protein S and the complement C4b-binding protein. *Seminars in Thrombosis and Hemost*asis 1984, **10**: 139-148.
9. Broze, G. J., *et al*. Biochemistry and Physiology of Protein C, Protein S, and Thrombomodulin. *In:* Colman, *et al.,* eds. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice, Third Edition* 1994. Philadelphia, PA: J. B. Lippincott Company, 259-275.
10. *Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition*, CLSI Document H21-A5, Vol. 28, No. 5, 2008.
11. Data on file at BioMedica Diagnostics Inc.
12. Boyer-Neumann, C., *et al*. Comparison of functional assays for Protein S. *Thrombosis and Haemostasis 1993*, **70**: 946-950.
13. How to Define and Determine Reference Intervals in the Clinical Laboratory – second edition. *NCCLS/CLSI Document C28-A2* June 2000; Vol. 20, No. 13
14. Bertina, R. M. Hereditary Protein S deficiency. *Haemostasis* 1995, **15**: 241-246.
15. Hathaway, W. E. and Goodnight, Jr., S. H. *Disorders of Hemostasis and Thrombosis: A Clinical Guide*. McGraw-Hill 1993, p. 350.

DEFINITIONS OF SYMBOLS

	Consult instructions for use		Warning
IVD	In vitro diagnostic medical device	2°C 8°C	Temperature limitation Store at 2°C to 8°C
LOT	Lot Number	REF	Catalog Number
	Expiration Date		Manufacturer
	Contains sufficient for <n> tests	CONT	Contains...
CE	CE mark	EC REP	European Union Authorized Representative