

Catalogue # 40345 (ROW) (4 x 10 Determinations)
Catalogue # 40390 (ROW) (2 x 10 Determinations)

BIOCLOT[®] PROTEIN S-300ACT

Test Kit for quantitative determination of Protein S Activity in human plasma using a Clotting End-Point.

For *in vitro* diagnostic use only.

FOR INFORMATION USE ONLY

Not to be used for performing the assay.

Refer to the insert accompanying kit

I. INTENDED USE

Bioclot[®] Protein S-300ACT is intended for the quantitative determination of Protein S activity in citrated human plasma using a clotting assay.

II. PRINCIPLE

Protein S is a Vitamin K-dependent plasma protein, which serves as a cofactor for the anticoagulant activity of activated Protein C in the degradation of Factors V and VIII ⁽¹⁾. The Bioclot[®] method is similar to a standard factor assay. Dilutions of normal plasma are mixed with Protein S depleted plasma. The mixed plasma is then activated by a reagent, which contains Factor Xa, activated Protein C, and phospholipid. After a five-minute activation time, clot formation is initiated by the addition of Calcium Chloride. Under these conditions, the prolongation of the clotting time is directly proportional to the concentration of Protein S in the patient plasma. The use of Factor Xa as the activator minimizes the potential interference by high levels of Factor VIII as found in some patients.

III. REAGENTS

A. Reagent Description

- Bioclot[®] Activator Reagent:**
1 ml, freeze-dried. Each vial contains human activated Protein C, Bovine Factor Xa, rabbit brain phospholipid, and Sodium Azide as a preservative.
- Protein S Depleted Plasma:**
1 ml, freeze-dried. Each vial contains human plasma that has been depleted of Protein S by immunoabsorption on a column of immobilized goat antibody to human Protein S.
- Sample Dilution Buffer (10x Concentrate):**
2.5 ml concentrate. Each vial contains buffer concentrate with Sodium Azide at 0.2%, which, when diluted, contains 0.2M NaCl, 0.03M Hepes, and 0.015M Sodium Azide at pH 7.35. The buffer also contains vPolybrene[®] sufficient to neutralize up to 1.2 U/ml heparin in the plasma sample.

v Polybrene[®] is a registered trademark of Abbott Laboratories.

B. Reagent Preparation

- Bioclot[®] Activator Reagent:** Reconstitute with 1 ml of reagent grade water. Let stand at room temperature (18-25°C) with occasional swirling for 20 minutes for complete dissolution. Store at 28°C and use within 4 hours. A standard curve must be prepared each time the assay is performed. Reconstituted Activator Reagent may be stored at -20°C for 30 days and thawed in an ice water bath prior to use.
- Protein S Depleted Plasma:** Reconstitute with 1 ml of reagent grade water. Let stand at room temperature (18-25°C) with occasional swirling for 20 minutes for complete dissolution. Store on ice or at 2-8°C and use within 4 hours. Plasma can be frozen at -20°C for 30 days.

- Sample Dilution Buffer:** Dilute contents of one vial to 25 ml with reagent grade water. Store at 2-8°C for 30 days.

IV. STORAGE AND STABILITY

All unreconstituted reagents are stable until the expiration date stated on the box and vial labels when stored at 2-8°C.

Do not use the component beyond use by date.

V. WARNINGS AND PRECAUTIONS

Each unit of source plasma used in the preparation of this product has been tested by FDA approved methods for the presence of antibody to Human Immunodeficiency Virus (HIV) Type I and Type II, Hepatitis B surface antigen (HB_sAg) as well as for Hepatitis C (HCV) and found negative (not repeatedly reactive). Donors have been screened for Creutzfeldt-Jakob Disease (CJD) and new variant Creutzfeldt-Jakob Disease (nvCJD) and found acceptable. However, no test can offer complete assurance that products derived from human blood will not transmit infectious disease. As with all materials of human origin, this product should be handled as a potentially infectious material. All wastes containing biological material should be properly labelled and stored separately from other wastes. Dispose of all waste materials according to prescribed international, national and local regulations.

Bioclot[®] Sample Dilution Buffer and Activator Reagent contain sodium azide as a preservative. Azides may react with lead or copper plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide buildup.

The test should be used in conjunction with clinical observations and results of other laboratory tests.

VI. SPECIMEN COLLECTION

- Obtain venous blood by clean venipuncture.
 - Immediately mix nine parts blood with one part anticoagulant, mix well by inversion of tube against the stopper.
 - Centrifuge the specimen at 1500 g for no less than 15 minutes.
 - Remove plasma from the tube within 60 minutes using a plastic pipette and store in a plastic tube.
 - Test plasma sample within 4 hours; otherwise, store frozen at -20°C for up to two weeks or -70°C for up to six months and thaw once just prior to use.
- (See NCCLS Standard H21-A3 ²⁾)

VII. PROCEDURE

A. Material Provided:

Bioclot[®] Activator Reagent
Protein S Depleted Plasma
Sample Dilution Buffer

B. Additional Material Required (but not provided):

- Clot timer or stopwatch
- Water bath at 37°C or dry bath
- Reagent Grade Water
- 0.025M Calcium Chloride solution (Biopool Catalogue #50355)
- 25 ml graduated cylinder
- 100-1000 µL variable volume pipetting device
- Graph paper
- Coagulation cuvette

NOTE: Procedure is also suitable for automated methods. Follow specific application for instrument in use.

C. Assay Procedure

- Reconstitute reagents as described in section III. **Reagents, B. Reagent Preparation.**
- Prepare plasma and standard dilutions as described below.
- Transfer Bioclot[®] Activator Reagent and Calcium Chloride to 37°C reagent wells in clot timer if instrument is manual. In the case of automated instruments, prime reagent delivery tubing,

set activation time to 5 minutes and maximum endpoint time to 150 seconds.

4. To a coagulation cuvette:
 - Add 0.1 ml Protein S Depleted Plasma + 0.1 ml test sample.
 - Incubate for 2 minutes at 37°C.
 - Add 0.1 ml Bioclot® Activator Reagent (pre-warmed to 37°C).
 - Incubate for exactly 5 minutes at 37°C.
 - Add 0.1 ml 0.025M Calcium Chloride (pre-warmed to 37°C).
 - Start timer and note time of clot formation.
 - Obtain duplicate determinations for each test dilution.

VIII. CALIBRATION

A. Assay Calibration

Pooled Normal Plasma (PNP), which has been collected in the same manner as plasma to be tested, should be used for preparation of Protein S calibration standards. At least 10 normal donors should be used to prepare this pooled standard. Commercially prepared reference plasma in which Protein S has been determined (e.g., Biopool Protein S Control Plasma, Catalogue #40225, Hemostasis Reference Plasma, Catalogue #50720) may also be used.

Prepare plasma Protein S calibration standards just before testing as follows:

Percent Protein S	Plasma	Sample Dilution Buffer
100% Standard	100 µl PNP	900 µl
50% Standard	500 µl 100% Standard	500 µl
25% Standard	500 µl 50% Standard	500 µl
12.5% Standard	500 µl 25% Standard	500 µl

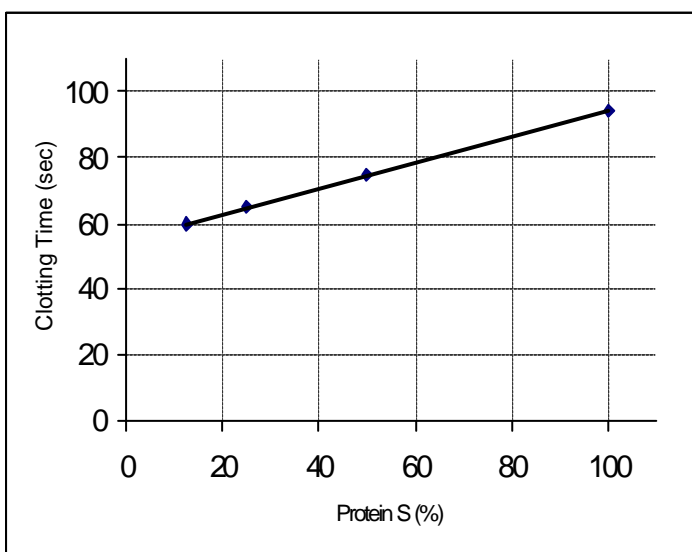
The patient samples are prepared by diluting 50 µl patient plasma with 450 µl Sample Dilution Buffer. Store dilutions at 2-8°C and test within 30 minutes.

B. Calibration Curve

Using graph paper, plot the % Protein S activity of the calibration standards on the x-axis vs. the mean clotting time on the y-axis. Draw the line of best fit between the resulting points. For patient samples determine the % Protein S by interpolating from the standard curve.

Sample Calibration Curve:

The calibration curve shown below is a sample only. Users must construct a standard curve each time the assay is performed. DO NOT USE THE SAMPLE CURVE BELOW.



IX. QUALITY CONTROL

Biopool Protein S Control Plasma (Catalogue #40225), Hemostasis Reference Plasma (Catalogue #50720), and Hemostasis Reference

Plasma Abnormal (Catalogue #50721) may be used for quality control. Controls should be included when freshly reconstituted reagents are used. The Protein S level obtained for each control should fall within the range specified in the directional insert for each plasma. If quality control samples fail to yield Protein S levels within these limits in any given run, the run should be repeated. Contact Trinity Biotech if controls still fail to yield Protein S levels within acceptable limits. If a reference plasma is used to construct the standard curve, a different lot of reference plasma must be used for quality control.

X. EXPECTED VALUES

The value for Protein S is usually expressed as percent compared to a standard or pooled normal plasma (100%). The expected normal range for Protein S is 55-160%⁽³⁾. A decrease in Protein S is associated with increased incidence of thromboembolism⁽⁴⁾.

A decrease in Protein S activity does not necessarily indicate a decrease in plasma concentration. Protein S (total) is present in plasma as free protein and as protein bound to C4B binding protein (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⁽⁵⁾, as summarized in the following table:

Deficiency Type	Total Protein S	Free Protein S	Protein S Activity
I	↓	↓	↓
IIa	=	↓	↓
IIb	=	=	↓

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

XI. LIMITATIONS AND INTERFERENCES

The presence of Heparin greater than 1.2 U/ml or Lupus-type anticoagulants may interfere with the assay results by prolonging the clotting time and hence giving an artificially high Protein S value.

In the case of patients with lupus anticoagulant or abnormally high Protein S activity, multiple dilutions should be tested and the Protein S level corrected for the dilution. If the corrected Protein S levels do not agree, the patient sample likely contains lupus-like anticoagulant activity and cannot be assigned an accurate activity level.

To assure accurate, reproducible results, use accurate pipetting devices and observe recommended procedures with particular emphasis on incubation times and temperature.

XII. PERFORMANCE CHARACTERISTICS

The user should establish product performance characteristics for the specific instrumentation used. Studies using an automated analyzer for photo-optical clot detection yielded the following results:

A. Precision

The following estimates of precision (coefficient of variation or CV) were observed:

Protein S Level	Within Assay CV	Between Assay CV
100%	8.3%	11.8%
50%	11.4%	13.0%

B. Accuracy

A study performed using another commercially available Protein S activity kit yielded a correlation coefficient of 0.8464, with a regression equation of $y = 0.83x + 15.9$.

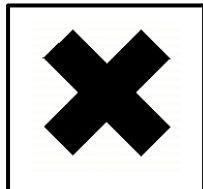
C. Sensitivity

Bioclot® Protein S is designed to give a linear standard curve for Protein S levels between 12.5 and 180% when plotted as directed.

D. Specificity

The specificity of the assay has been established in studies employing plasma that has been selectively depleted of Protein S followed by addition of purified Protein S to achieve various concentrations.

XIII. RISK AND SAFETY



Harmful – Sodium Azide

R22 Harmful if Swallowed.

R32 Contact with acids liberates toxic gas.

S36 Wear suitable protective clothing.

XIV. REFERENCES

1. Walker, F.J., Protein S and the Regulation of Activated Protein C, *Semin. Thromb. Hemost.*, 10:131-138, 1984.
2. National Committee for the National Laboratory (NCCLS) Standards: Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, Document H21-A2, vol. 11, No. 23, 1991.
3. Boyer-Neumann, C. *et al*, Comparison of Functional Assays for Protein S, *Thromb. Hemost.*, 70:946-950, 1993.
4. Bertina, R.M., Hereditary Protein S Deficiency, *Haemostasis*, 15:241-245, 1985.
5. Comp, P.C., Laboratory Evaluation of Protein S Status, *Semin. Thromb. Hemost.*, 16:177-181, 1990.

XV. KEY GUIDE TO SYMBOLS



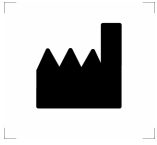
Use by



Lot



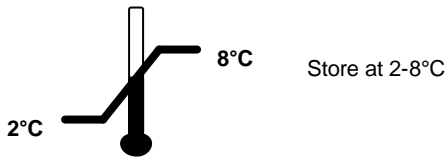
Catalogue number



Manufacturer



For *in vitro* diagnostic use



Consult accompanying documents



Biological risks

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