HYPHEN BioMed
155 rue d'Eragny, 95000 Neuville-sur-Oise, France
Tél : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
www.hyphen-biomed.com
info@hyphen-biomed.com

CE IVD

HEMOCLOT™ Protein S

REF CK042K R1 R2 3 x 2 mL

Clotting method for the measurement of Protein S activity

English, last revision: 07-2024

INTENDED USE:

The HEMOCLOT™ Protein S kit is a clotting method for the *in vitro* quantitative determination of Protein S (PS) activity on citrated human plasma, using a manual or automated method.

SUMMARY AND EXPLANATION:

Technical:

Protein S (PS) is a vitamin K dependent protein, mainly synthesized in liver. Plasma PS exists in two forms: complexed with C4b-BP, or as free form that presents anticoagulant activity by acting as cofactor of Activated Protein C (APC). In the presence of calcium and phospholipids, the APC-PS complex inhibits Factors Va and VIIIa.[1]

Clinical context of the test:

The PC/PS inhibition pathway has decreased activity if PS free form is deficient or abnormal. Congenital or acquired PS deficiencies are associated with an increased risk of venous thrombosis.[1,2]

PS activity is age and sex dependent [3,4], and may be decreased in various contexts such as: Vitamin K deficiency or VKA treatment (predisposing more to bleeding than thrombotic problems), L-asparaginase therapy, hepatic disorders, nephrotic syndrome, during pregnancy, related to oral contraceptives intake or oestrogen therapy, early stages of inflammatory diseases, viral infections, disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT), pulmonary embolism (PE), rarely due to acquired or transient anti-PS autoantibodies (e.g. in children with chickenpox).[1,2]

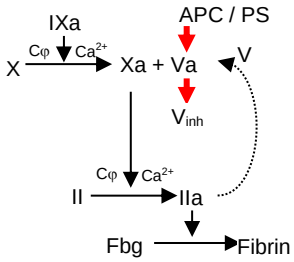
Inherited protein S deficiencies are classified into three types: type I and III deficiencies account for 95% of cases of PS deficiency.[1,5]

Mutated Factor V (eg FV Leiden mutation R506Q) is resistant to the inactivation of its coagulant function by the APC-PS complex. [1]

PRINCIPLE:

The HEMOCLOT™ Protein S kit is a clotting method, using activated partial thromboplastin time (APTT), triggered by Factor IXa in the presence of phospholipids, calcium and a constant and in excess amount of APC.

The diluted test plasma is mixed with PS deficient plasma (R1). The activator reagent (R2), at constant and optimized concentration, is added. Coagulation is triggered by adding calcium (Ca^{2+}). Since PS is the limiting factor, this results in a direct relationship between PS concentration and the corresponding measured clotting time.



REAGENTS:

R1 Protein S deficient plasma, immuno-depleted, lyophilized in the presence of an heparin neutralizing agent.

R2 Activator reagent, lyophilized. Contains human Factor IXa, human APC, and phospholipids, in an optimized concentration. Contains BSA.

REF CK042K ➔ R1 R2 3 vials of 2 mL

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R1 R2 Reconstitute the contents of each vial with exactly:

REF CK042K ➔ 2 mL of distilled water

Shake vigorously until complete dissolution while avoiding formation of foam and load it on the analyzer following application guide instruction.

For manual method, allow to stabilize for 15 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R1 R2 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **24 hours** at 2-8°C.
- **8 hours** at room temperature (18-25°C).
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific application.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water
- Imidazole Buffer (AR021B/AR021K/AR021L/AR021M/AR021N)
- CaCl2 at 0.025M (AR001B/AR001K/AR001L)
- Specific calibrators and controls:

Product name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301

Use the same buffer for all tests performed.

Also refer to the specific application guide of the analyzer used.

Materials:

- Water-bath, semi-automatic or automatic analyzer for clotting assays.
- Stopwatch; Calibrated pipettes; silicon glass or plastic test tubes, stir bar (27425/89174) for use with automatic analyzer STA-R® family

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[6] guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references [6,7].

PROCEDURE:

The kit can be used in manual or automated method. The assay is performed at **37°C**, and the clotting time, triggered by the addition of calcium, is measured.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay method:

1. Reconstitute the calibrator and controls as indicated in the specific instructions. The calibrator should be diluted in Imidazole buffer as described below in order to perform the calibration range ("C" defines the PS concentration or by definition 100% for a normal plasma pool):

When calibration is performed with a commercially available plasma calibrator (e.g. BIOPHEN™ Plasma Calibrator), the **1:10** dilution corresponds to the indicated "**C**" PS activity concentration.

For a calibrator titrating **C**, the level of 100% (in the assay conditions) is obtained by diluting this standard by the following factor: **10x(C)/100**.

The calibration range can also be performed using a pool of normal citrated plasma (at least 30 normal individuals, men and women, aged 18 to 55, with no known treatment or pathology), which by definition is at **100%** of PS. The assay incorporates a **1:10** dilution of the plasma, which corresponds by definition to 100% of PS. The calibration range is **0 to 100%** PS.

Prepare **3 mL** of the **1:10** dilution of the normal pool of plasmas, or a dilution **(10xC/100)** of the calibrator plasma titrated in PS (i.e. **C1**). This solution corresponds to 100% of PS. Prepare the following calibration range by successive dilutions in the Imidazole buffer as described in the table below in order to perform the calibration range:

Calibrator	C5	C4	C3	C2	C1
Protein S (%)	**0%**	**25%**	**50%**	**75%**	**100%**
Volume of calibrator	0 mL	0,250 mL	0,500 mL	0,750mL	1 mL
Volume of imidazole bufer	1 mL	0,750 mL	0,500 mL	0,250mL	0 mL

2. Dilute the samples in imidazole buffer as described in the table below:

Samples	Reference	Dilution
Control	223201/223301	**1/10**
Samples	N.A.	**1/10**

For best results, for expected concentrations >100%, values can be obtained by testing plasma at 1:20 dilution and then multiply the results by 2; for sample ≤10%, use 1:5 dilution and divide the result by 2.

Realize the calibration range and test it quickly with the quality controls. Diluted samples should be tested quickly, if stored at room temperature (18-25°C). Whenever possible, for optimal performance, all tests (calibration, samples and controls) should be carried out extemporaneously and simultaneously. The exact concentrations of the calibrators and controls are indicated for each lot on the flyer supplied with the kit.

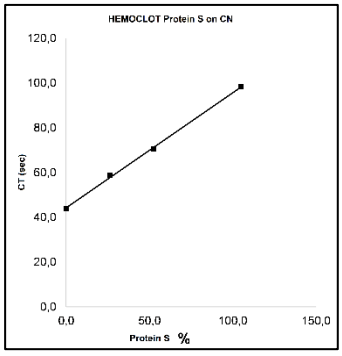
3. In a plastic tube incubated at **37°C**, introduce:

	Volume
Calibrator, control or plasma (diluted)	50 µL
R1 **Protein S deficient plasma,** preincubated at **37°C**	50 µL
Mix and incubate for **1 minute at 37°C,** then add:	
R2 **Activator reagent,** preincubated at **37°C**	50 µL
Mix and incubate for **3 minutes at 37°C,** then add (starting the stopwatch):	
$CaCl_2$ 0.025M (preincubated at **37°C**, and stirred)	100 µL
Note the clotting time, in seconds	CT

If a reaction volume different from that indicated above is required for the method used, the volume ratio must be strictly observed in order to guarantee the performance of the assay. The user is responsible for validating changes and their impact on all results.

CALIBRATION:

The HEMOCLOT™ Protein S assay can be calibrated for the functional assay of plasma Protein S. The calibrator covering the calibration range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.
The calibration range is about 0 to 100% (on CN series).
The calibration curve shown below is given by way of example only. The calibration curve established for the assay series must be used.



QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual end point method, plot the calibration curve lin-lin, with the clotting time (sec) along the Y-axis and the PS concentration, expressed as %, along the X-axis.
- The concentration of PS (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.

The results should be interpreted according to the patient's clinical and biological condition.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- The test can be performed in patients treated with heparin (up to 1 IU/mL) or on VKA (PS activity is reduced). Special attention should be paid to known patients with abnormal, high levels of FVIII:C, Lupus anticoagulant (LA), or mutated FV (R506Q, FV Leiden). Aprotinin tending to inhibit activated Protein C, the "apparent" PS activity may be diminished in aprotinin treated patients [8]. If clotting times are abnormally shortened or prolonged, the result should be confirmed by another method (e.g. immunological) and / or another sample, and considered according to the clinical context.
- For a same batch of reagents, and a same plasma, the clotting time (CT) may vary depending on the instrument used (particularly depending on the detection of the clot in mechanical or optical mode) and the adjustment of the clot detection sensitivity.

EXPECTED VALUES:

The normal plasma Protein S level in adult population is usually in the range 60 to 140% (variable according to age and sex) [3,4]. However, each laboratory has to determine its own normal range.

PERFORMANCES:

Performances studies were conducted as described in CLSI guidelines.
The following performance data represent typical results and are not to be regarded as specifications for HEMOCLOT™ Protein S.
Mathematical analyses are performed using a validated statistical software built in accordance with CLSI guidelines.
All performances are documented in the respective Application Guides of the analyzers.

Analytical performances
Measuring Range
The measuring range is defined by the analyzer system used and is documented in the respective Application Guides of the analyzers.
Accuracy
Accuracy studies were assessed using laboratory controls and pooled plasmas.
Trueness: bias is less than 13% for all samples.
Precision: coefficient of variation (CV) for all samples is less than 7% or repeatability, less than 10% for reproducibility and less than 8% for within laboratory. Precision is documented in the respective Application Guides of the instruments.
Interfering substances
Interferences are defined by the analyzer system used and are documented in the respective Application Guides of the analyzers.

Clinical performances

Agreement

	ACL TOP® family			
Analyte	**n**	**Linear regression**	**r**	**Reference / comparison method**
Protein S	129	y = 0.92x+8.56	0.940	HemosIL® Protein S

Sensitivity/Specificity

	ACL TOP® family				
Analyte	**n**	**Sensitivity**	**Specificity**	**Area under the curve (ROC)**	
Protéine S	129	0.97	0.97	0.998	
Analyte	**n**	**PPV**	**NPV**	**LR+**	**LR-**
Protéine S	129	97%	98%	31.99	0.02

PPV: Predictive value of a positive result LR+ : Likelihood Ratio +
NPV: Predictive value of a negative result LR- : Likelihood Ratio –

REFERENCES:

1. Meireles Rezende S. *et al.* Coagulation, inflammation, and apoptosis : different roles for protein S and the protein S – C4b binding protein complex. Blood. 2004.
2. Wypasek E. and Undas Anetta. Protein C and Protein S Deficiency – Pratical Diagnostic Issues. Adv Clin Exp Med. 2013.
3. Appel IM et al, Age dependency of coagulation parameters during childhood and puberty, J Thromb Haemost. 2012.
4. Lowe GDO et al, Epidemiology of coagulation factors, inhibitors and activation markers: the Third Glasgow MONICA survey I. Illustrative reference ranges by age, sex and hormone use. Br J Haematol. 1997.
5. Castoldi E. *et al.* Similar hypercoagulable state and thrombosis risk in type I and type III protein S-deficient individuals from families with mixed type I/III protein S deficiency. Haematologica. 2010.
6. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
7. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.
8. Tanaka K.A. et *al*, The effect of aprotinin on activated protein C-mediated downregulation of endogenous thrombin generation. Br J Haematol. 2006.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to previous version.