Free Protein S - 0020002700

ENGLISH - Insert revision 04/2004

CE

Intended use

Automated latex ligand immunoassay for the quantitative determination of free Protein S in human citrated plasma on IL Coagulation Systems.

Summary and principle

Protein S is a vitamin K-dependent cofactor for the anticoagulant and the profibrinolytic effects of activated Protein C.[1] Two forms of Protein S are present in plasma: free Protein S (40%), and Protein S linked to the complement C4b-binding protein (C4BP) (60%). Only free Protein S has functional cofactor activity.[2]
Protein S deficiency may be hereditary[3] or acquired. Acquired deficiency may be observed during pregnancy, oral anticoagulant therapy, oral contraceptive use, in liver disease, in newborn infants as well as in other clinical conditions.[4,5,6] Deficiency of Protein S has been associated with a high risk of developing venous thromboembolism especially in young people.[7,8]
The presence of free Protein S is determined by measuring the increase of turbidity produced by the agglutination of two latex reagents. Purified C4BP adsorbed onto the first latex reagent reacts with a high affinity for free Protein S of patient plasma in the presence of Ca^{2+} ions.[9,10,11] The free Protein S adsorbed on the C4BP latex triggers the agglutination reaction with the second latex reagent which is sensitized with a monoclonal antibody directed against human Protein S. The degree of agglutination will be directly proportional to the free Protein S concentration in the test sample.

Composition

The **Free Protein S** kit consists of:

- **B** **C4BP Buffer** (Cat. No. 0020002720): 3 vials x 4 mL of Borax buffer containing bovine serum albumin, stabilizers and preservative.
- **R1** **C4BP Latex** (Cat. No. 0020002710): 3 vials x 4 mL of a lyophilized suspension of polystyrene latex particles coated with purified human C4BP containing bovine serum albumin, stabilizers and preservative.
- **R2** **Anti PS MAb Latex** (Cat. No. 0020002730): 3 vials x 2 mL of a suspension of polystyrene latex particles coated with a monoclonal antibody directed against human Protein S containing bovine serum albumin, stabilizers and preservative.

PRECAUTIONS AND WARNINGS:
The material in this product was tested by FDA approved test methods and found nonreactive for Hepatitis B Surface Antigen (HBsAg), Anti-HCV and HIV 1/2 antibodies. Handle as if potentially infectious.[12]
Avoid contact with skin and eyes (S 24/25). Do not empty into drains (S 29). Wear suitable protective clothing (S 36).

This product is For *in vitro* Diagnostic Use.

Preparation

C4BP Buffer: The reagent is ready for use.
C4BP Latex: Dissolve the contents of each vial of C4BP latex by pouring the entire contents of one vial of C4BP buffer into the latex reagent vial. Replace the stopper and swirl gently for a minimum of 20 seconds to completely dissolve the lyophilized latex. Make sure of the complete reconstitution of the product. It must appear as a homogenous and slightly milky suspension. Keep the reagent at 15-25°C for 30 minutes and invert to mix before use. Do not shake.
Anti PS MAb Latex: Invert to mix before use. Do not shake.

Note: Avoid foam formation when homogenizing reconstituted reagents. Bubbles on top of the liquids may interfere with the instruments liquid sensors.

Reagent storage and stability

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.

C4BP Latex - Stability after reconstitution: 1 month at 2-8°C in the original vial, 1 week at 15°C on the ACL® 8000/9000/10000, ACL Futura®/ACL Advance Systems and ACL TOP™. Do not freeze.

Anti PS MAb Latex - Opened reagent is stable 1 month at 2-8°C in the original vial, 1 week at 15-25°C on the ACL 8000/9000/10000 and 1 week at 15°C on the ACL Futura/ACL Advance Systems and ACL TOP. Do not freeze.

For optimal stability remove reagents from the system and store them at 2-8°C in the original vial.

Instrument/test procedures

Refer to the appropriate IL instrument's Operator's Manual and/or Application Manual for the complete assay procedure instructions.

Specimen collection and preparation

Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Refer to NCCLS Document H21-A4 for further instructions on specimen collection, handling and storage.[13]
Frozen plasma samples should be rapidly thawed at 37°C while gently mixing before testing. After thawing the assay must be performed within 2 hours.

Additional reagents and control plasmas

The following are not supplied with the kit and must be purchased separately.

	Americas and Pacific Rim Cat. No.	Europe Cat. No.
Calibration plasma	0020000000	0008467300
Normal Control	0020003120	0020003110
Special Test Controls Level 1 & 2	0008467600	0008467600
Low Abnormal Control	0020003220	0020003210
High Abnormal Control	0020003320	0020003310
Factor diluent	0009757600	0009757600

Quality control

Normal and abnormal controls are recommended for a complete quality control program.[14] Normal Control, Low Abnormal Control, High Abnormal Control and Special Test Controls Level 1 & 2 are designed for this program. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance to good laboratory practice. Refer to the instrument's Operator's Manual for additional information. Refer to Westgard *et al* for identification and resolution of out-of-control situtations.[15]

Results

Free Protein S results are reported in % normality. Refer to the instrument's Operator's Manual for additional information. The assay results should be used with other information, including the clinical context, in forming a diagnosis.

Limitations/interfering substances

Free Protein S results on the ACL and ACL Futura/ACL Advance Systems are not affected by heparin (UF heparin or LMW heparin) up to 1.5 IU/mL, bilirubin up to 18 mg/dL, hemoglobin up to 200 mg/dL, lipids up to 1280 mg/dL, platelets up to 10^{11}/L and rheumatoid factor up to 350 IU/mL.
Free Protein S results on the ACL TOP are not affected by heparin (UF heparin or LMW heparin) up to 1.5 IU/mL, bilirubin up to 25 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 1500 mg/dL, platelets up to 10^{10}/L and rheumatoid factor up to 900 IU/mL.
Free Protein S assay is not affected by Factor V Leiden mutation (APC-R).

Expected values

A normal range study was performed using the Free Protein S kit on IL Coagulation Systems.

Sex	N	ACL 8000/9000/10000	ACL Futura ACL Advance	N	ACL TOP
Male (% free PS)	130	72.2 - 123.3	64.4 - 128.8	128	74.1 - 146.1
*Female (% free PS)	102	57.6 - 112.5	53.2 - 109.1	128	54.7 - 123.7

* Note: Age and hormonal status may affect the normal range for females.[16] Ranges were calculated as recommended by the International Federation of Clinical Chemistry (IFCC).[17] These results were obtained using a specific lot of reagent. Due to many variables which may affect results, each laboratory should establish its own free Protein S normal range.

Performance characteristics

Precision:
Within run and total (run to run and day to day) precision was assessed over multiple runs.

ACL 8000/9000/10000	Mean (% free PS)	CV% (Within run)	CV% (Total)
Normal Control	97.1	1.8	3.0
Special Test Controls Level 1	56.6	2.9	4.9
Special Test Controls Level 2	21.8	3.2	5.9

ACL Futura ACL Advance	Mean (% free PS)	CV% (Within run)	CV% (Total)
Normal Control	97.7	1.9	2.4
Special Test Controls Level 1	57.0	2.7	3.1
Special Test Controls Level 2	22.4	2.9	3.4

ACL TOP	Mean (% free PS)	CV% (Within run)	CV% (Total)
Normal Control	112.0	2.5	3.4
Special Test Controls Level 1	64.9	1.9	3.2
Special Test Controls Level 2	29.1	2.9	4.2

Correlation:

System	slope	intercept	r	Comparative method
ACL 8000/9000/10000	0.933	5.09	0.972	Free Protein S EIA
ACL Futura/ACL Advance	0.918	2.32	0.981	Free Protein S EIA
ACL TOP	1.000	-7.0	0.986	Free Protein S on ACL Advance

The precision and correlation results were obtained using specific lots of reagents and controls.

Detection limit:

System	
ACL 8000/9000/10000	3.2 (% free Protein S)
ACL Futura/ACL Advance	6.4 (% free Protein S)
ACL TOP	7.3 (% free Protein S)

Linearity:

System	
ACL 8000/9000/10000	10 - 135 (% free Protein S)
ACL Futura/ACL Advance	12 - 135 (% free Protein S)
ACL TOP	11 - 150 (% free Protein S)

If the linear range is exceeded samples should be manually diluted 1:2 with Factor diluent (200 µL of sample + 200 µL of Factor diluent) and reassayed. The printed results must be multiplied by 2 to correct for the dilution. Instruments with Auto Rerun capability perform the 1:2 dilution and result correction automatically.

Tests on IL Coagulation Systems

Free Protein S	75 tests (approx.)

Instrumentation Laboratory

Instrumentation Laboratory Company - Lexington, MA 02421-3125 (USA)
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Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Χρησιμοποιηθέντα σύμβολα

IVD	LOT				CONTROL			EC REP
In vitro diagnostic medical device	Batch code	Use by	Temperature limitation	Consult instructions for use	Control	Biological risks	Manufacturer	Authorised representative
In-vitro Diagnostikum	Chargen-Bezeichnung	Verwendbar bis	Festgelegte Temperatur	Beilage beachten	Kontrollen	Biologisches Risiko	Hergestellt von	Bevollmächtigter
De uso diagnóstico *in vitro*	Identificación número de lote	Caducidad	Temperatura de Almacenamiento	Consultar la metódica	Control	Riesgo biológico	Fabricado por	Representante autorizado
Dispositif mèdical de diagnostic *in vitro*	Désignation du lot	Utilisable jusqu'à	Températures limites de conservation	Lire le mode d'emploi	Contrôle	Risque biologique	Fabricant	Mandataire
Per uso diagnostico *in vitro*	Numero del lotto	Da utilizzare prima del	Limiti di temperatura	Vedere istruzioni per l'uso	Controllo	Rischio biologico	Prodotto da	Rappresentanza autorizzata
Dispositivo médico para utilização em diagnóstico *in vitro*	Número de lote	Data límite de utilização	Límite de temperatura	Consultar as instruções de utilização	Controlo	Risco biológico	Fabricado por	Representante autorizado
"in vitro" diagnostisk udstyr	Batch nr.	Anvendelse	Temperatur begrænsninger	Se vejledning for anvendelse	Kontrol	Miljø oplysninger	Producent	Leverandør
In vitro diagnostisk medicinsk produkt	Tillverkningskod	Användning	Temperatur gräns	Ta del av instruktionen före användning	Kontroll	Biologiska risker	Tillverkare	Auktoriserad representant
In vitro	Αρ. Παρτίδας	Χρήση έως	Περιορισμοί θερμοκρασίας	Συμβουλευτήτε τις οδηγίες χρήσης	Υλικό ποιοτικού ελέγχου	Βιολογικοί κίνδυνοι	Κατασκευαστής	Εξουσιοδοτημένος αντιπρόσωπος
Προϊόν για διαγνωστική χρήση								