Product information

User's Manual

Free Protein-S ELISA

Enzyme Immunoassay for the detection of
free Protein-S in citrated human plasma

REF AE29030

Σ 96

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

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1 INTENDED USE

This Free Protein-S ELISA is a solid phase enzyme immunoassay for the determination of only free Protein-S in citrated human plasma.

For research use only, not for use in diagnostic procedures.

2 INTRODUCTION

Protein-S is a vitamin K dependent glycoprotein of 70 kDa that is mainly synthesized by hepatocytes, but also by endothelial cells, Leydig cells in the testis, and megakaryocytes. In human plasma it is present at a concentration of 25 µg/ml and has a half-life of approximately two days. About 40 % of Protein-S circulates in a functionally active free form, whereas 60 % is complexed with C4b-binding protein. Protein-S plays an essential role in the Protein-C anticoagulant system where the free Protein-S functions as a cofactor of activated Protein-C (aPC). Among the vitamin K dependent proteins Protein-S has the highest affinity for negatively charged phospholipids and therefore increases the affinity of activated Protein-C to membranes by forming a complex. This is of physiological importance since aPC inactivates preferentially the membrane-bound coagulation factors Va and VIIIa. Protein-S deficiency may be inherited or acquired and increases the risk of thrombotic events such as deep vein thrombosis, pulmonary embolism, or thrombophlebitis. The prevalence of Protein-S deficiency has been estimated to be up to one case per 300 in the general population. Nearly 50 % of individuals with inherited Protein-S deficiency will experience a thrombotic event before the age of 45. Acquired Protein-S deficiency occurs more frequently than the inherited form. Amongst others it can be found during oral anticoagulant therapy, oral contraceptive, pregnancy, liver disease, diabetes mellitus, chemotherapy and various inflammatory syndromes. Protein-S deficiency is classified in three states. Type I deficiency is a reduction in the level of both Free and Total Protein-S. Type II deficiency is characterized by a reduced Protein-S activity, with normal antigen level. Type III deficiency corresponds to reduced antigen level and activity of free Protein-S only. To determine the type of defect, the laboratory diagnosis of Protein-S may require antigen levels of both free and total Protein-S and functional determination.

3 PRINCIPLE OF THE TEST

Principle of the test

This test is using microplates coated with a capture antibody specific for human free Protein-S. Diluted plasma samples (1:51) are incubated in the wells allowing free Protein-S present in the plasma to bind to the antibody. The unbound fraction is removed by washing. Afterwards anti-human Protein-S detection antibody conjugated to horseradish peroxidase (conjugate) is incubated and reacts with the antigen-antibody complex on the microwell surface. Following incubation, unbound conjugate is washed off. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is measured in optical density units with a spectrophotometer at 450 nm. Using a curve prepared from the Reference Plasma provided with the kit, the free Protein-S antigen relative percent concentration in the plasma samples can be determined.

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4 REAGENTS

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
Reference Plasma	3 x 0,4ml	White	-	lyophilized human plasma
Control N	3 x 0,2ml	White	-	lyophilized human plasma
Control D	3 x 0,2ml	White	-	lyophilized human plasma
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Conjugate, IgG	1 x 15ml	Blue	Blue	anti-human free Protein-S antibody conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized TMB/H_2O_2
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to chapter 3 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

5 STORAGE CONDITIONS

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

6 WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[25]
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For research use only, not for use in diagnostic procedures.

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7 INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

8 SAMPLE COLLECTION AND PREPARATION

Use preferentially plasma samples freshly collected with 3.2% or 3.8% sodium citrate as an anticoagulant. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Blood samples should be collected in clean, dry and empty tubes. After centrifugation, the plasma samples should be used immediately, otherwise stored tightly closed at 2-8°C/35-46°F up to eight hours, or frozen at -20°C/-4°F for longer periods.

9 PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel). Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

10 PREPARATION OF REAGENTS AND SAMPLES

All reagents should be brought to room temperature (18 °C - 25 °C) before use.

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml). Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Reference Plasma:

Reconstitute Reference Plasma by adding 0.4 ml distilled water and shake gently. Allow the reconstituted plasma to stand for 10 minutes at room temperature before use. The Reference Plasma is stable for 8 hours when stored at 2-8°C/35-46°F.

Controls:

Reconstitute Control N and Control D by adding 0.2 ml distilled water and shake gently. Allow the reconstituted Controls to stand for 10 minutes at room temperature before use. The Controls are stable for 8 hours when stored at 2-8°C/35-46°F.

Predilution of the Reference Plasma:

Prepare a 1:2 dilution of reconstituted reference plasma in prediluted sample buffer (1x) and mix well, e.g. 100 µl sample buffer + 100 µl plasma.

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Preparation of the reference curve:

The dilution set is prepared by using the prediluted Reference Plasma.

Volume Reference Plasma	Volume Sample Buffer	Reference Level
60 µl	1000 µl	150 %
40 µl	1000 µl	100 %
30 µl	1000 µl	75 %
20 µl	1000 µl	50 %
10 µl	1000 µl	25 %
10 µl	2000 µl	12.5 %

Dilution of the Samples and Controls:

Add 20 µl plasma to 1000 µl sample buffer (1x) and mix well.

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells (e.g. 4 ml concentrate plus 196 ml distilled water).

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

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11 ASSAY PROCEDURE

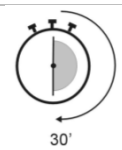
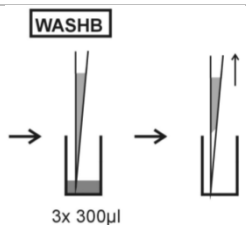
We suggest pipetting calibrators, controls and samples as follows:

for quantitative interpretation use the working dilutions of the Reference Plasma to establish a standard curve

	1	2	3	4...	
A	150	25	P1		
B	150	25	P1		
C	100	12.5	P2		
D	100	12.5	P2		
E	75	CD	P3		
F	75	CD	P3		
G	50	CN	...		
H	50	CN	...		

150: Reference Level 150 %
100: Reference Level 100 %
75: Reference Level 75 %
50: Reference Level 50 %
25: Reference Level 25 %
12.5: Reference Level 12.5 %
CD: control ‚deficient plasma
CN: control ‚normal plasma'
P1: sample 1
P2: sample 2
P3: sample 3

12 TEST STEPS

Step	*Description*
1.	Ensure preparations from step 10 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with the intended quantitative interpretation of the results:
	Calibrators, controls, and samples
3.	 • Pipette 100 µl of each diluted sample plasma into the designated microwells. • Pipette 100 µl of each working dilution of the Reference Plasma and the diluted Controls into the designated wells.
4.	 Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	 Wash 3x in each case with 300 µl washing buffer (diluted 1:50).

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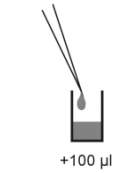
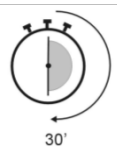
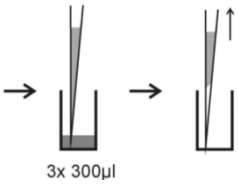
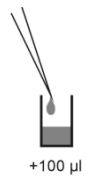
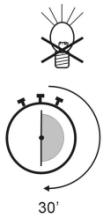
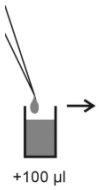
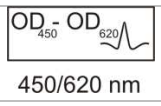
	CONJUGATE	
6.	CONJ 	Pipette 100 µl conjugate into each well.
7.		Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.	WASHB 	Wash 3x in each case with 300 µl washing buffer (diluted 1:50).
	SUBSTRATE	
9.	SUB 	Pipette 100 µl TMB substrate into each well.
10.		Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
	STOP	
11.	STOP 	Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.	 450/620 nm	Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

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13 INTERPRETATION

For **quantitative interpretation** establish the reference curve by plotting the optical density (O.D.) of each dilution of the Reference Plasma (y-axis) against the corresponding value of the Reference Level in % (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the O.D. of each sample, read the corresponding sample relative value expressed in %. Multiply the sample relative value obtained from the reference curve by the assigned factor referred in the quality control leaflet to calculate the free Protein-S antigen level in % of normal.

Example of a standard curve

We recommend pipetting each dilution of the Reference Plasma in parallel for each run.
Do NOT use this example for interpreting the sample results!

Reference Level	OD 450/620 nm	Results (%)	CV % (Variation)
12.5 %	0.433	11.98	4.16
25 %	0.754	23.45	6.20
50 %	1.275	53.63	7.26
75 %	1.581	76.53	2.04
100 %	1.881	99.71	0.29
150 %	2.371	146.52	2.32

Example of calculation

Sample	Replicate (OD)	Mean (OD)	Sample relative value (%)	Factor	Conc. Protein S (%)
P 01	0.933/0.927	0.930	29.5	0.96	28.32
P 02	1.860/1.866	1.863	123.5	0.96	118.56

Samples that are above the highest calibrator value should be reported as > max. They should be diluted accordingly and be re-evaluated, taking the dilution factor into account. Samples lower than the measurement range should be reported as < min.

For batch-specific data please see the attached QC certificate. Medical laboratories should perform in-house quality controls with their own controls and/or pooled sera according to national legislation.

It is recommended that each laboratory works out its own normal values, based on its own technology, controls, equipment and population.

If the control values do not meet the validation criteria, the test is invalid and must be repeated.

The following technical data should be reviewed: expiry dates of the reagents, storage conditions, pipettes, used equipment, photometer, incubation conditions and washing methods.

If the tested samples reveal unusual values or deviations, or if the validation criteria are not met for inexplicable reasons, please contact the IBL-America.

Expected values

The values for free Protein-S are given in relative percent (%) as compared to pooled normal plasma. The free Protein-S concentration in normal human plasma ranges usually between 60 % and 130 %. Samples with values above the range of the reference curve may be assayed again at higher dilutions for accurate results.

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14 TECHNICAL DATA

Sample material:	Plasma
Sample volume:	20 µl plasma diluted 1:51 with 1x sample buffer
Total incubation period:	90 minutes at 20-26°C/68-78.8°F.
Calibration range:	12.5-150 %
Analytical sensitivity:	1,0%
Storage:	at 2-8°C/35-46°F in original bottles.
Number of determinations:	96 tests

15 PERFORMANCE DATA

15.1 Analytical Sensitivity

Testing sample buffer 30 times with this Free Protein-S ELISA gave an analytical sensitivity of 1.0 %.

15.2 Specificity

The microplate is coated with an antibody specific for human free Protein-S.

15.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly.

Sample No.	Dilution Factor	Measured %	Expected %	Recovery (%)
1	1 / 50	97.66	100	97.66
	1 / 100	49.51	50	99.02
	1 / 200	25.66	25	102.64
	1 / 400	13.36	12.5	106.88
2	1 / 50	42.97	40	107.43
	1 / 100	18.78	20	93.90
	1 / 200	9.78	10	97.80
	1 / 400	4.85	5	97.0

15.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the reference curve.

Intra-assay		
Sample No.	**Mean %**	**CV (%)**
1	110	2.3
2	78	5.6
3	26	4.2

15.5 Calibration

This Protein-S ELISA is calibrated against the WHO second international standard for Protein-S. The values are given in relative percent (%) as compared to pooled normal plasma.

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16 LITERATURE

Murdock PJ, Brooks S, Mellars G, Cheung G, Jacob D, Owens DL, Parmar M, Riddell A (1997). A simple monoclonal antibody based ELISA for free protein S. Comparison with PEG precipitation. Clinical and Laboratory Haematology 19: 111-114.

Deutz-Terlouw PP, Ballering L, van Wijngaarden A, Bertina RM (1989). Two ELISA's for measurement of protein S, and their use in the laboratory diagnosis of Protein S deficiency. Clinica Chimica Acta 186: 321-334.

Persson KEM, Hillarp A, Dahlbäck B (2001). Analytical considerations for free protein S assays in protein S deficiency. Thrombosis and Haemostasis 86: 1144-1147.

Walker FJ (1984). Protein S and the regulation of activated protein C. Seminars in Thrombosis and Hemostasis 10: 131-138.

Preissner KT (1990). Biological relevance of the Protein C system and laboratory diagnosis of Protein C and S deficiencies. Clinical Science 17: 351-364.

Manufactured for :
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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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