ZYMUTEST™ Total Protein S (II)

REF RK021B-RUO

96 tests

ELISA method for the quantitative determination of Total Protein S.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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

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INTENDED USE:

ZYMUTEST™ Total Protein S (II) kit is an ELISA method for the *in vitro* quantitative determination of Total Protein S in human plasma.
This kit is for research use only and should not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:
Protein S concentration in normal human plasma is about 25 µg/mL[1]. About 40% (i.e. 10 µg/mL) is in the free form and 60% (i.e. 15 µg/mL) circulates in blood as a non-covalent complex with C4b-BP[2]. Only the free form has an anticoagulant activity as the cofactor of Activated Protein C.
Protein S is synthesized in liver. It is a vitamin K dependent glycoprotein, with a molecular weight of 80,000 daltons. The balance between the free form and the C4b-BP bound form of protein S plays an important role because only the Free Protein S is active. In the early stages of inflammatory diseases, Free Protein S concentration is decreased as a result of an elevation of C4b-BP. Protein S is decreased in dicoumarol or L-asparaginase therapy, and in hepatic diseases.

PRINCIPLE:

The ZYMUTEST™ Total Protein S (II) assay is specific for both forms of Protein S (free or complexed with C4b-BP) and is designed with a rabbit polyclonal antibody.
First, the immunoconjugate, a rabbit polyclonal antibody specific for both forms of Protein S (free or complexed with C4b-Binding Protein) coupled to horse radish peroxidase (HRP), is introduced into the microwells coated with the same rabbit polyclonal antibody. Then, the diluted tested plasma is immediately introduced, and the immunological reaction starts. When present, Protein S (free or complexed with C4b-BP) binds onto the polyclonal antibody coated solid phase through epitopes and fixes the polyclonal antibody coupled to HRP by other epitopes. Following a washing step, the peroxidase substrate, 3,3',5,5' – Tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H_2O_2), is introduced and a blue colour develops. When the reaction is stopped with sulfuric acid, a yellow colour is obtained that can be read at 450nm. The absorbance at 450nm is directly proportional to the concentration of Total Protein S in the sample.

REAGENTS:

1. **COAT ELISA Microplate**, 12x8 containing 12 strips of 8 wells, coated with a polyclonal antibody specific for the two forms of human protein S, stabilized and packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD ELISA Sample Diluent:** 2 vials of 50 mL, ready to use. Contains BSA and mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1).
3. **CAL PROTEIN S Plasma Calibrator:** 3 vials 2 mL, lyophilized. Each vial must be reconstituted with **2 mL** of **SD ELISA** to obtain a plasma already diluted **1:50**. Contains BSA.
4. **CI PROTEIN S Control I (High):** 1 vial of 0.5 mL, lyophilized. Contains BSA.
5. **CII PROTEIN S Control II (Low):** 1 vial of 0.5 mL, lyophilized. Contains BSA.
6. **IC Anti-(h)-Total Protein S (II)-HRP immunoconjugate:** 3 vials of 4 mL, Polyclonal antibody coupled to HRP, lyophilized. Contains BSA and 4-Aminobenzoic acid sodium salt.
7. **CD ELISA Conjugate Diluent:** 1 vial of 25 mL, ready to use. Contains BSA and mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1).
8. **WS ELISA Wash Solution:** 1 vial of 50 mL, 20x 20 fold concentrated. Contains mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1).
9. **TMB 3,3', 5,5' – Tetramethylbenzidine:** 1 vial of 25 mL peroxidase substrate, ready to use.
10. **Stop 0.45M Sulfuric Acid:** 1 vial of 6 mL, ready to use. Contains 0.45M sulfuric acid.

The calibrator and controls concentrations may vary slightly from one batch to the next. For the assay, see the exact values provided on the flyer provided with the kit used.
The calibrator and controls are titrated relative to a Reference Internal Standard.

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.
- Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Wear protection glasses and gloves when handling. Avoid any skin and eye contact.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- If the TMB substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device is intended for professional use in the laboratory for research only.

CD **SD** **WS** H317 : May cause an allergic skin reaction.

REAGENT PREPARATION:

Allow the strips and reagents to stabilize for at least 30 min at room temperature before use. Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

COAT Open the aluminum pouch and take off the required amounts of strips for the test series. The strips must be used within 30 minutes.

Reconstitute the contents of each vial with exactly:
CAL PROTEIN S ➔ **2 mL of SD ELISA in order to obtain a solution containing** "C" % **of Total Protein S (already diluted at 1:50)**. Shake vigorously until complete dissolution.
CI PROTEIN S ➔ **0.5 mL of distilled water**. Shake vigorously until complete dissolution.
CII PROTEIN S ➔ **0.5 mL of distilled water**. Shake vigorously until complete dissolution.
IC ➔ **4 mL of CD ELISA** at least 15 minutes before use. Let the pellet to be completely dissolved and shake gently until complete dissolution.

SD ELISA **TMB** **Stop** **CD ELISA**
Reagent ready to use.

WS ELISA Shake the vial and dilute the wash solution **1:20 in distilled water** (the 50 mL of concentrated solution allow to prepare 1 litter of wash solution after dilution).
Incubate, if necessary, the vial in a water bath at **37°C**, until complete dissolution of solids.

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.

COAT Unused strips can be stored at 2-8°C **for 4 weeks** in their original aluminum pouch (hermetically closed, in presence of the desiccant), stored in the provided plastic microplate storage bag (minigrip), protected from any moisture.

Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:
CAL PROTEIN S
➔ **72 hours** at 2-8°C.
8 hours at room temperature 18-25°C.
CI PROTEIN S **CII PROTEIN S**
➔ **24 hours** at 2-8°C.
8 hours at room temperature 18-25°C.
2 months frozen at -20°C or less*.
IC
➔ **4 weeks** at 2-8°C.
24 hours at room temperature 18-25°C.
*Thaw only once, as rapidly as possible at 37°C and use immediately.

Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:
SD ELISA **CD ELISA** **TMB** **Stop**
➔ **4 weeks** at 2-8°C.
WS ELISA ➔ **4 weeks** at 2-8°C.
7 days at 2-8°C for the diluted solution.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:
- Distilled water.

Materials:
- 8-channel or repeating pipette allowing dispensing volumes of 50-300 µL.
- 1-channel pipettes at variable volumes from 0 to 20 µL, 20 to 200 µL and 200 to 1000 µL.
- Micro ELISA plate washing equipment and shaker.
- Micro ELISA plate reader with a wavelength set up at 450 nm.
- For variant protocol: Dissociation Buffer (AR035K-RUO).

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 GP44-A4[3] (and CLSI H21-A5[4]) guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references.

PROCEDURE:

I. Assay method:

1. Specimens and controls should be diluted using **SD ELISA** as described in the table below:

Specimens	Dilution
CI PROTEIN S and **CII PROTEIN S**	1:50
Specimens	1:50

In presence of specimen with high amounts of Protein S, dilute at **1:100** or more. The obtained results should be multiplied by **2** or more. For low amounts of Protein S levels (<10%) the sample can be tested at a lower dilution. The obtained results should be divided by the dilution factor.

2. Using the **Calibrator** **CAL PROTEIN S** with a concentration "**C**" in %, prepare the calibration range as described in the table below:

Total Protein S concentration (%)	C	C:2	C:4	C:10	C:20	0
Vol. of **CAL PROTEIN S**	1 mL	0.5 mL	0.25 mL	0.1 mL	0.05 mL	0 mL
Vol. of **SD ELISA**	0 mL	0.5 mL	0.75 mL	0.9 mL	0.95 mL	1 mL

Mix for homogenization.
The standard dilutions are stable for **4 hours** at room temperature (18-25°C).

3. Put strips in the frame provided. Introduce the reagents in the micro ELISA plate wells and perform the assay as indicated on the following table:

Reagent	Volume	Procedure
IC	**100 µL**	Introduce the **IC** into the plate wells.
CAL PROTEIN S or **CI PROTEIN S** or **CII PROTEIN S** or **Specimens to test diluted** or **SD ELISA (blank)**	**100 µL**	Introduce **immediately** the standard solutions or controls or the tested specimen in the corresponding micro ELISA plate well.
Mix gently then incubate for 1 hour at room temperature (18-25°C) (a)		
WS ELISA	**300 µL**	Proceed to 5 successive washings **(b)**
TMB	**200 µL**	Immediately after the washing, introduce the substrate into the wells **(a, b, c).** **Nota:** The substrate distribution, raw by raw, must be accurate and at exact time intervals.
Incubate for exactly 5 minutes at room temperature (18-25 °C) (a).		
Stop	**50 µL**	Following exactly the same time intervals, raw to raw, than for the addition of substrate, stop the colour development by introducing the 0.45M sulfuric acid **(c)**.
Wait for 10 minutes in order to allow the color to stabilize then measure absorbance at 450 nm. Subtract the blank values (d).		

Distribute calibrator dilutions, controls and specimens as rapidly as possible, in order to obtain homogeneous kinetics of the dosage. A too long delay (>10 min) between the first and the last distribution wells may have incidence on immunological kinetics and produce inaccurate results (underestimated value for the last wells).

(a) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro ELISA plate shaker can be used.

(b) Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized components and reduce the reactivity plate. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could damage coating and lower plate reactivity.

(c) For addition of the substrate, the time interval between each row must be accurate and exactly determined.

(d) For bichromatic readings, a reference wavelength at 620 nm or at 690 nm can be used.

QUALITY CONTROL:

Using quality controls allows validating the method compliance, as well as the homogeneous of assays for a same lot of reagents.
Quality control plasmas must be included in each series, as per good laboratory practice, in order to validate test results. A new calibration curve must be carried out for each test series.
Each laboratory can establish acceptance ranges and verify expected performances in its analytical system.

RESULTS:

- Plot the calibration curve with the OD 450 nm along the Y-axis and the Total Protein S concentration in %, along the X-axis by choosing the "best fit" interpolation mode (refer to the flyer in the kit).
- The concentration of Total Protein S (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution **1:50** is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.
- Alternatively, a specific software (i.e., Dynex, Biolise, etc...) can be used for the calculation of concentrations.
- When employing the kinetic method, use ΔOD 450 instead of OD 450.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

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 total protein S assay is lower because the conformation of the complexes makes the protein S less accessible to antibodies [5,6] .

PERFORMANCES:

The ZYMUTEST™ Total Protein S (II) kit is specific for both forms of Protein S (free or complexed with C4b-BP). The polyclonal antibodies bind as well as to Free Protein S as to complexes with C4b-BP.

- Dynamic range: 5 to about 130%.
- Detection threshold ≤ 2%.
- Intra-assay CV ≤ 8%.
- Inter-assay CV ≤ 10%.
- Interferences: No interference was observed with the molecules and up to following concentrations:

Heparin	Bilirubin	Hemoglobin
1 IU/mL	0.2 mg/mL	2 mg/mL

REFERENCES:

1. Faioni E. *at al*. Free Protein S Deficiency is a Risk Factor for Venous Thrombosis. Thromb. Haemost. 1997.
2. Henkens C.AA. *et al*. Plasma Levels of Protein S, Protein C, and Factor X. Effects of sex, Hormonal State and Age : Thromb. Haemost. 1995.
3. CLSI Document GP44-A4: "Procedures for the handling and processing of blood specimens for common laboratory tests".
4. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
5. Dahlbäck B. Protein S and C4b-binding protein: components involved in the regulation of the protein C anticoagulant system. Thromb Haemost. 1991.
6. Dahlbäck B. Vitamin K-Dependent Protein S: Beyond the Protein C Pathway. Semin Thromb Hemost. 2018.

SYMBOLS:

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

HYPHEN BioMed
155 rue d'Eragny, 95000 Neuville-sur-Oise France