Human Protein S ELISA Kit

Enzyme Immunoassay for the quantification of total and free Protein S in citrated human plasma.

Catalog number: ARG81038

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Protein S is a vitamin K-dependent plasma glycoprotein synthesized in the liver. In the circulation, Protein S exists in two forms: a free form and a complex form bound to complement protein C4b-binding protein (C4BP). In humans, protein S is encoded by the PROS1 gene.

The best characterized function of Protein S is its role in the anti-coagulation pathway, where it functions as a cofactor to Protein C in the inactivation of Factors Va and VIIIa. Only the free form has cofactor activity.

Protein S testing can be used to diagnose thrombosis caused by congenital or non-congenital protein S deficiency, such as pregnancy and liver disease. [Provide by Wikipedia: Protein S]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique.

A capture antibody specific for Human Protein S has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any human protein S present is bound by the immobilized antibody. An HRP conjugated antibody specific for Human Protein S is added to each well and incubate. After washing away any unbound antibody, the substrate solution (TMB substrate) is added to the wells and color develops in proportion to the amount of Human Protein S bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm.The concentration of human protein S in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Store microplates in designated foil, including the desiccant, and seal tightly. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
PEG solution	2 mL X 2	4°C
5X Diluent Buffer	20 mL	4°C
HRP-Conjugate antibody (Anti-Human protein S antibody)	15 mL (ready to use)	4°C
50X Washing Buffer Concentrate	20 mL	4°C
TMB substrate	15 mL (ready to use)	4°C (protect from light)
STOP solution	15 mL (ready to use)	4°C
Reference Plasma	3 vials (lyophilized)	4°C
Control N (Normal human plasma)	3 vials (lyophilized)	4°C
Control D (Deficient human plasma)	3 vials (lyophilized)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (600-690 nm as optional reference wave length)
- Deionized or distilled water
- Vortex mixer
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Stored at 2-8°C the reconstitution the Reference Plasma and the Controls are stable for at 8 hours or frozen at-20°C for longer periods.
- Briefly spin down the all vials before use.
- If crystals are observed in the 50X Wash buffer and 5X Diluent buffer, warm to room temperature (20-26°C) until the crystals are completely dissolved.
- Never expose components to higher temperature than 37°C.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Avoid microbial contamination of reagents and equipment. Automated

plate washers can easily become contaminated thereby causing assay variability.

- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, do NOT use it.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Plasma: Collect blood with 3.2% or 3.8% sodium citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Note:

1. Do not use haemolytic, icteric, lipaemic or bacterially contaminated specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Aliquot samples for testing and store at -20°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent buffer as necessary.
4. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (E.g., add 10 ml of 50X Wash buffer into 490 ml of distilled water to a final volume of 500 ml) The 1X Washing Buffer is stable for up to 4 weeks when stored at 2-8°C.
- 1X Diluent Buffer: Dilute 5X Diluent buffer into distilled water to yield 1X Diluent buffer. (E.g., add 10 ml of 5X Diluent buffer into 40 ml of distilled water to a final volume of 50 ml) The 1X Diluent buffer is stable for up to 4 weeks at 2-8°C.
- 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.
- 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.
- Pre-treatment with polyethylene glycol (PEG) for Free Protein S determination: Do not dilute plasma samples before PEG pretreatment. Add 15 µl of PEG solution to 85 µl patient plasma or Controls. To prepare the reference curve add 45 µl of PEG solution to 255 µl of the reconstituted Reference Plasma. Vortex the samples and place them on ice for 30 mins. Following incubation centrifuge the samples for 10 minutes at 3000 x g. Prepare the reference curve, the Control dilution and the sample dilution by using the supernatant as described as follows.

- **Pre-dilution of the Reference Plasma for Total and Free Protein S determination**: For Total Protein S the pre-dilution is prepared by using the reconstituted Reference Plasma. For Free Protein S the pre-dilution is prepared by using the supernatant of the PEG-treated Reference Plasma. Prepare a 1:2 dilution of each reference plasma in 1X Diluent buffer and mix well, e.g. 100 µl Diluent buffer + 100 µl plasma.
- **Reference curve:** Separate reference curves are used for Total and Free Protein S assays. The dilution sets are prepared by using the pre-dilutions of the Reference Plasma for Total and Free Protein S, respectively.

Volume Reference Plasma	Volume of Diluent 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**:

  For Total Protein S: Add 20 µl plasma to 1000 µl 1X Diluent buffer and mix well.

  For Free Protein S: Add 20 µl of supernatant of the PEG-treated plasma to 1000 µl 1X Diluent buffer and mix well.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-26°C) before use. Standards, samples and controls should be assayed in duplicates.

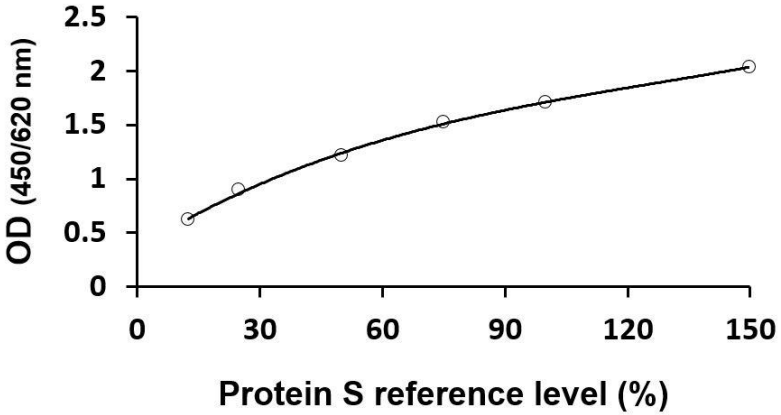
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µL of sample or working dilution of the Reference Plasma to the Antibody-coated microplate.
3. Incubate at RT for 30 mins on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µL of the HRP conjugated antibody to each well. Incubate at RT for 30 mins on a microplate shaker.
6. Aspirate each well and wash as step 3
7. Add 100 µl of TMB Substrate to each well. Incubate for 30 minutes at RT in the dark.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
8. Add 100 µl of Stop Solution to each well. Incubate for 5 minutes and agitate plate carefully for 5 sec. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately. (optional:

read at 620 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with Human Protein S ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Us the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 1.1-2.9% and inter-assay precision was 4.7-9.8%.

Sensitivity

Testing sample buffer 30 times on PROTEIN S gave an analytical sensitivity of 1.0 %.