

ab125969

Human Protein S

ELISA Kit

Instructions for Use

For the quantitative measurement of Human Protein S in plasma, serum, cell culture supernatants, cell lysates and tissue samples.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

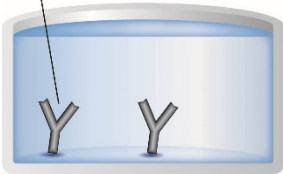
Abcam's Protein S Human *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Human protein S in plasma, serum and cell culture supernatants.

A Protein S specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently biotinylated Protein S is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is inversely proportional to the amount of Protein S captured in plate.

Protein S is a single-chain vitamin K-dependent plasma glycoprotein consisting of 635 amino acids with a molecular weight of about 70 kDa. It functions as a cofactor for the anticoagulant activated protein C to inhibit blood coagulation. In plasma, it is both in a free cofactor active form and in an inactive form complexed with C4b-binding protein (C4BP). The complex acts as a bridge between coagulation and inflammation due to the involvement of C4BP in regulating complement activation. Hereditary protein S deficiency causes recurrent venous thrombosis. Acquired protein S deficiency is associated with nephrotic syndrome, disseminated intravascular coagulation, liver disease and the use of oral anticoagulants, leading to increased thrombotic risk.

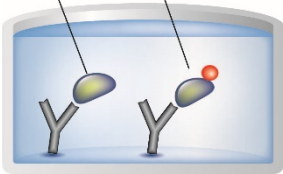
2. ASSAY SUMMARY

Primary Capture Antibody



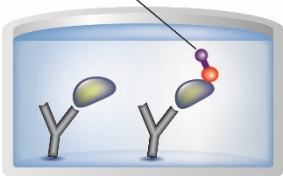
Prepare all reagents, samples and standards as instructed.

Sample Biotinylated Antigen



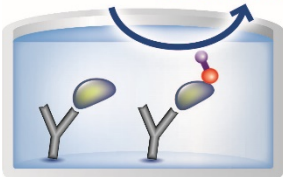
Add standard or sample to each well used and add prepared biotin protein to each well. Incubate at room temperature.

Streptavidin-HRP



Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.

Substrate Colored Product



Wash and add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Protein, which should be stored at -20°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Protein S Microplate (12 x 8 well strips)	96 wells	4°C
Protein S Standard (lyophilized)	1 vial	4°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated Human Protein S (Lyophilized)	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	30 mL	4°C
Sealing Tapes	3	N/A

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 μ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 7 tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

9.1 1X Diluent N

Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

9.2 1X Wash Buffer

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

9.3 1X Biotinylated Protein S

Add 4 mL 1X Diluent N to the lyophilized Biotinylated Protein S vial to generate a 2X Biotinylated Protein S stock solution. Allow the vial of 2X Biotinylated Protein S stock solution to sit for 10 minutes with gentle agitation prior to use. The stock solution should be further diluted 1:2 with 1X Diluent N to generate 1X Biotinylated Protein S.

Any remaining solution should be frozen at -20°C.

9.4 1X SP Conjugate

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N

Any remaining solution should be frozen at -20°C.

10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
- This procedure prepares sufficient standard dilutions for duplicate wells.

10.1 Reconstitution of the Protein S Standard vial to prepare the 8 µg/mL Protein S **Standard #1**.

10.1.1 First consult the Protein S Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the Protein S Standard vial to produce an 8 µg/mL Protein S **Standard #1** by using the following equation:

C_S = Starting mass of Protein S Standard (see vial label) (µg)

C_F = The 8 µg/mL Protein S **Standard #1** final required concentration

V_D = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_F) \times 1,000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 9.6 µg of Protein S Standard in vial

C_F = 8 µg/mL Protein S **Standard #1** final concentration

V_D = Required volume of 1X Diluent N for reconstitution

$$(9.6 \mu\text{g} / 8 \mu\text{g/mL}) \times 1,000 = 1,200 \mu\text{L}$$

- 10.1.3 First briefly spin the Protein S Standard vial to collect the contents on the bottom of the tube.
- 10.1.4 Reconstitute the Protein S Standard vial by adding the appropriate calculated amount V_D of 1X Diluent N to the vial to generate the 8 $\mu\text{g}/\text{mL}$ Protein S **Standard #1**. Mix gently and thoroughly.
- 10.2 Allow the reconstituted 8 $\mu\text{g}/\text{mL}$ Protein S **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions.
- 10.3 Label six tubes #2-7.
- 10.4 Add 120 μL of 1X Diluent N to tubes #2 – 7.
- 10.5 To prepare **Standard #2**, add 120 μL of the **Standard #1** into tube #2 and mix gently.
- 10.6 To prepare **Standard #3**, add 120 μL of the **Standard #2** into tube #3 and mix gently.
- 10.7 Using the table below as a guide, prepare subsequent serial dilutions.
- 10.8 1X Diluent N serves as the zero standard, 0 $\mu\text{g}/\text{mL}$ (tube #7).

ASSAY PREPARATION

Standard Dilution Preparation Table

Standard #	Volume to Dilute (μL)	Volume Diluent N (μL)	Total Volume (μL)	Starting Conc. ($\mu\text{g}/\text{mL}$)	Final Conc. ($\mu\text{g}/\text{mL}$)
1	Step 10.1				8.000
2	120	120	240	8.000	4.000
3	120	120	240	4.000	2.000
4	120	120	240	2.000	1.000
5	120	120	240	1.000	0.500
6	120	120	240	0.500	0.250
7	-	120	120	-	0



11. SAMPLE PREPARATION

11.1 Plasma

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x *g* for 10 minutes. Dilute samples 1:10 into 1X Diluent N and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant).

11.2 Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x *g* for 10 minutes and remove serum. Dilute samples 1:10 into 1X Diluent N. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatant

Centrifuge cell culture media at 3,000 x *g* for 10 minutes to remove debris. If necessary, dilute samples into 1X Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

11.4 Cell Lysate

Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 10⁶ cells, add approximately 100 µl of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. If necessary, dilute samples into MIX Diluent; user should determine optimal dilution factor depending on application

needs. The undiluted samples can be stored at -80°C . Avoid repeated freeze-thaw cycles.

11.5 Tissue

Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into MIX Diluent; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C . Avoid repeated freeze-thaw cycles.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18-25°C) prior to use.**
 - **It is recommended to assay all standards, controls and samples in duplicate.**
- 13.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).
 - 13.2 Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
 - 13.3 Add 25 μL of Protein S Standard or sample per well, and immediately add 25 μL of Biotinylated Protein S to each well (on top of the standard or sample). Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
 - 13.4 Wash five times with 200 μL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 μL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid..
 - 13.5 Add 50 μL of 1X SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
 - 13.6 Wash microplate as described above.

- 13.7 Add 50 μL of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 20 minutes or till the optimal blue colour density develops.
- 13.8 Add 50 μL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
- 13.9 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at low concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

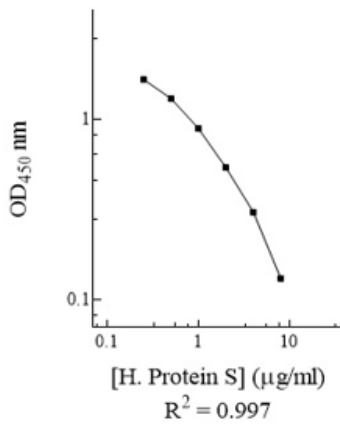
14. CALCULATIONS

Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Human Protein S Standard Curve



16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of Protein S is typically 0.4 µg/mL.

RECOVERY –

Standard Added Value: 1 – 4.0 µg/mL

Recovery %: 93 - 113.

Average Recovery %: 97

LINEARITY OF DILUTION –

Plasma Dilution	Average % Expected Value
1:5	103
1:10	101
1:20	93

Serum Dilution	Average % Expected Value
1:5	104
1:10	98
1:20	92

PRECISION –

	Intra- Assay	Inter- Assay
% CV	6.2	9.0

17. ASSAY SPECIFICITY

Species	% Cross Reactivity
Canine	< 50
Mouse	None
Monkey	< 20
Bovine	None
Rat	None
Swine	< 20
Rabbit	None
Equine	25

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

RESOURCES

Problem	Cause	Solution
High background/ Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

19. NOTES

Technical Support

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