

# **ab137988**

## **Human Protein C**

### **ELISA Kit**

#### Instructions for Use

For the quantitative measurement of Human Protein C in plasma and serum.

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

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## 1. BACKGROUND

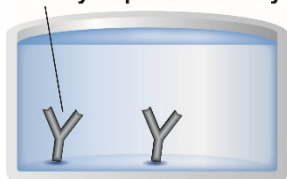
Abcam's Protein C Human *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Protein C in plasma and serum.

A Protein C specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently biotinylated Protein C 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 C captured in plate.

Protein C is a vitamin K-dependent plasma antithrombotic and anti-inflammatory zymogenic glycoprotein that is synthesized in the liver. Protein C has a light chain of 155 amino acids (21 kDa) and a heavy chain of 262 amino acids (41 kDa) linked by a disulfide bond. On endothelial cell membrane, thrombin-thrombomodulin complex cleaves a 12-residue peptide from protein C amino terminus of the heavy chain and converts it to activated protein C (APC). APC inactivates coagulation Factor Va and Factor VIIIa and performs a major role in regulating blood clotting, inflammation, and apoptosis.

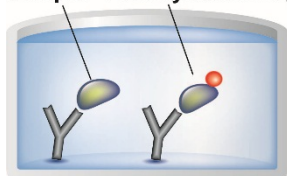
## 2. ASSAY SUMMARY

### Primary Capture Antibody



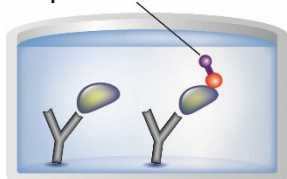
Prepare all reagents, samples and standards as instructed.

### Sample Biotinylated Antigen



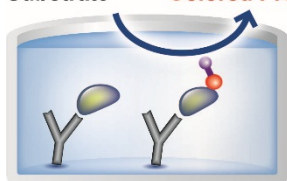
Add standard or sample to each well used and add prepared biotin protein to each well. Incubate at room temperature.

### 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 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 C Microplate (12 x 8 well strips)	96 wells	4°C
Protein C Standard	1 vial	4°C
10X Diluent M Concentrate	20 mL	4°C
Biotinylated Human Protein C (Lyophilized)	1 vial	4°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  $\mu$ 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.
- 8 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. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

### 9.1 1X Diluent M

Dilute the 10X Diluent M 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 C

Reconstitute the Biotinylated Human Protein C Protein with 4 ml of 1X Diluent M concentrate to generate a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 15 days.**

### 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 M.

*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 15 days.
- This procedure prepares sufficient standard dilutions for duplicate wells.

10.1 Reconstitution of the Protein C Standard vial to prepare a 6 µg/mL Protein C **Standard #1**.

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

10.1.2 Calculate the appropriate volume of 1X Diluent M to add when resuspending the Protein C Standard vial to produce a 6 µg/mL Protein C **Standard #1** by using the following equation:

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

$C_F$  = The 6 µg/mL Protein C **Standard #1** final required concentration

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

Calculate total required volume 1X Diluent M 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.**

$C_S$  = 2.7 µg of Protein C Standard in vial

$C_F$  = 6 µg/mL Protein C **Standard #1** final concentration

$V_D$  = Required volume of 1X Diluent M for reconstitution

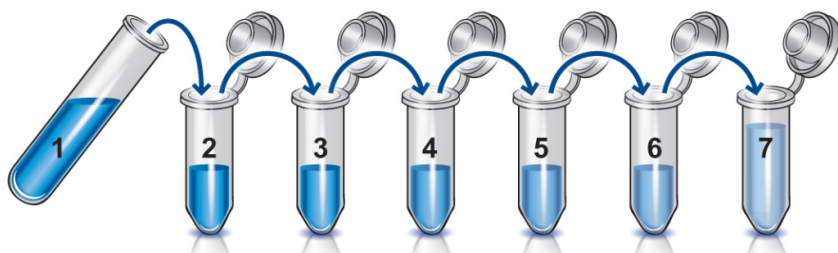
$$(2.7 \mu\text{g} / 6 \mu\text{g/mL}) \times 1,000 \mu\text{L} = 450 \mu\text{L}$$

- 10.1.3 First briefly spin the Protein C Standard Vial to collect the contents on the bottom of the tube.
- 10.1.4 Reconstitute the Protein C Standard vial by adding the appropriate calculated amount  $V_D$  of 1X Diluent M to the vial to generate the 6 µg/mL Protein C **Standard #1**. Mix gently and thoroughly.
- 10.2 Allow the reconstituted 6 µg/mL Protein C **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- 10.3 Label seven tubes #2 - 8.
- 10.4 Add 120 µL of 1X Diluent M to tubes #2 – 8.
- 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 M serves as the zero standard, 0 µg/mL (tube #8).

# ASSAY PREPARATION

**Standard Dilution Preparation Table**

Standard #	Volume to Dilute (μL)	Volume Diluent M (μL)	Total Volume (μL)	Starting Conc. (μg/mL)	Final Conc. (μg/mL)
1	Step 10.1				6.000
2	120	120	240	6.000	3.000
3	120	120	240	3.000	1.500
4	120	120	240	1.500	0.750
5	120	120	240	0.750	0.375
6	120	120	240	0.375	0.188
7	120	120	240	0.188	0.094
8	-	120	120	-	0



## 11. SAMPLE PREPARATION

### 11.1 **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 1:8 into 1X Diluent M and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

### 11.2 **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 1:8 into 1X Diluent M 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 an anticoagulant).

## 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 C Standard or sample per well, and immediately add 25 µL of Biotinylated Protein C to each well (on top of the standard or sample) and tap plate to mix gently. 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 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 and incubate for about 15 minutes or till the optimal blue colour density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

## ASSAY PROCEDURE

- 13.8 Add 50  $\mu$ L of Stop Solution to each well. The color will change from blue to yellow.
- 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**

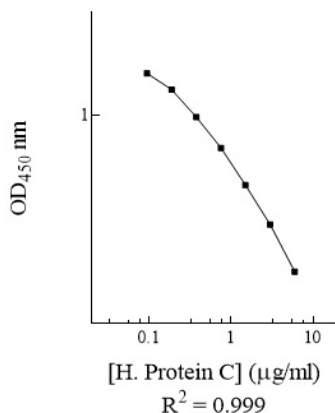
Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log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



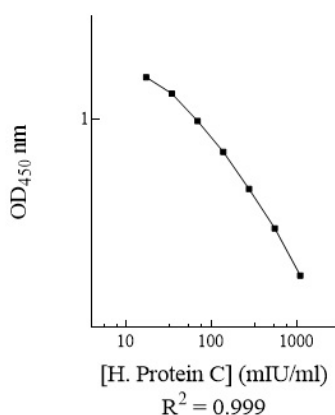
## 15. TYPICAL DATA

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

Human Protein C Standard Curve



Human Protein C Standard Curve



## 16. TYPICAL SAMPLE VALUES

### SENSITIVITY –

The minimum detectable dose of Protein C is typically 76 ng/mL.

### SPIKING RECOVERY –

Recovery was determined by spiking one plasma and one serum sample with different protein C concentrations.

Sample	Unspiked Sample (ng/ml)	Spiking Value (ng/ml)	Expected	Observed	Recovery (%)
Plasma	0.572	1.448	2.020	1.880	93
		0.347	0.919	0.965	105
		0.067	0.639	0.695	109
Serum	0.494	1.448	1.942	1.800	93
		0.347	0.841	0.820	98
		0.067	0.561	0.532	95
Average recovery					99

### REFERENCE VALUES

The normal blood level of Protein C level ranges from 3 to 5 µg/mL.

Plasma and serum samples from healthy adults were tested (n=20).

On average, human protein C level was 3.9 µg/ml.

## LINEARITY OF DILUTION –

Plasma Dilution	Average % Expected Value
1:4	90
1:8	100
1:16	110

Serum Dilution	Average % Expected Value
1:4	106
1:8	93
1:16	101

## PRECISION –

	Intra-Assay	Inter-Assay
% CV	5.1	10.7



**17. ASSAY SPECIFICITY**

<b>Species</b>	<b>% Cross Reactivity</b>
Bovine	20
Monkey	30
Mouse	5
Rat	10
Swine	80
Rabbit	None
Canine	10
Equine	50

## 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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**For all technical or commercial enquiries please go to:**

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