PROC (Human) ELISA Kit

Catalog Number KA1840

96 assays

Version: 02

Intended for research use only

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Table of Contents

Introduction

Background

Protein C (PROC) 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-reside 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 (1-3). Protein C deficiency causes neonatal purpura fulminans, thrombophilia, and recurrent venous thrombosis (4-6). Protein C pathway components have been studied in the treatment of complex disorders, including severe sepsis, thrombosis, and ischemic stroke (7).

Principle of the Assay

The PROC (Human) ELISA Kit is designed for detection of human Protein C in urine, saliva, milk, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Protein C in less than 4 hours. A polyclonal antibody specific for human Protein C has been pre-coated onto a 96-well microplate with removable strips. Protein C in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for Protein C, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Human Protein C Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Protein C.	96(8x12) wells
Sealing Tapes: Precut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human Protein C Standard: Human Protein C in a buffered protein base (lyophilized).	400 ng
Biotinylated Protein C Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Protein C.	80 µL
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate.	80 µL
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction.	12 mL

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channels).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acidic solution.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- ✓ Standard Curve: Reconstitute the 400 ng of Protein C Standard with 2 mL of MIX Diluent to generate a solution of 200 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (200 ng/mL) 1:2 with equal volume of MIX Diluent to produce 100, 50, 25, 12.5, 6.25 and 3.125 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	**Dilution**	**[Protein C] (**ng/mL**)**
P1	Standard (200 ng/mL)	200.0
P2	1 part P1 + 1 part MIX Diluent	100.0
P3	1 part P1 + 1 part MIX Diluent	50.00
P4	1 part P3 + 1 part MIX Diluent	25.00
P5	1 part P4 + 1 part MIX Diluent	12.50
P6	1 part P5 + 1 part MIX Diluent	6.250
P7	1 part P6 + 1 part MIX Diluent	3.125
P8	MIX Diluent	0.000

- ✓ Biotinylated Human Protein C Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Sample Preparation

- ✓ Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:20 into MIX Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- ✓ Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples

1:800 into MIX Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- ✓ Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:6000 into MIX Diluent Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
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.
3. Add 50 µL of Human Protein C standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
5. Add 50 µL of Biotinylated Protein C Antibody to each well and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
11. 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 high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

Calculation of Results

- ✓ Calculate the mean value of the duplicate or 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.
- ✓ The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

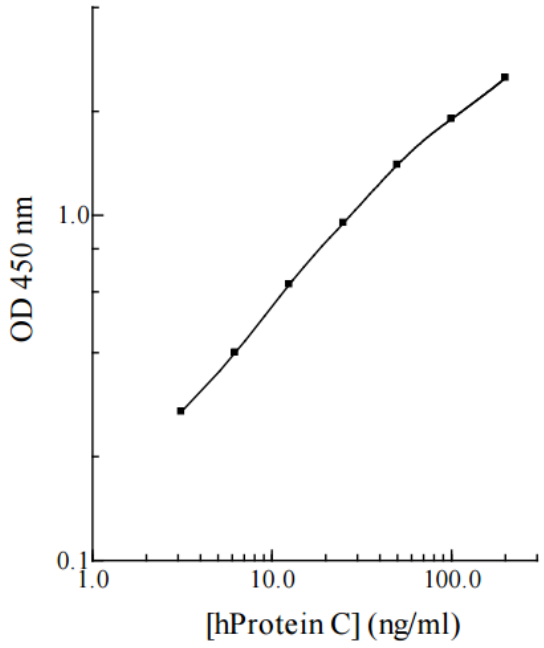

Figure 1: Typical Standard Curve for PROC (Human) ELISA Kit

Performance Characteristics

- ✓ The minimum detectable dose of Protein C is typically ~3 ng/mL.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.8% and 7.3% respectively.

✓ Linearity

	Average Percentage of Expected Value
Sample Dilution	Urine
1:10	89%
1:20	96%
1:40	93%

	Average Percentage of Expected Value
Sample Dilution	Milk
1:3000	94%
1:6000	99%
1:12000	98%

	Average Percentage of Expected Value
Sample Dilution	Saliva
1:400	94%
1:800	99%
1:1600	98%

✓ Recovery

Standard Added Value	5 – 50 ng/mL
Recovery %	87-109 %
Average Recovery %	98 %

✓ Cross-Reactivity

Species	% Cross Reactivity
Canine	20%
Bovine	None
Monkey	90%
Mouse	5%
Rat	1%
Swine	5%
Rabbit	None

Resources

References

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Plate Layout

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