Datasheet for ABIN612753

PROC ELISA Kit

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Overview

Quantity:	96 tests
Target:	PROC
Reactivity:	Human
Method Type:	Competition ELISA
Minimum Detection Limit:	0.9 µg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Protein C ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Protein C in plasma and serum
Brand:	AssayMax
Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Reference Value: The normal blood level of Protein C1 is ~4 µg/ml.
Components:	Human Protein C Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Protein C. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Human Protein C Standard: Human Protein C in a buffered protein base (6 µg, lyophilized). Biotinylated Protein C: 1 vial, lyophilized. EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered

Product Details

	surfactant (30 ml). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80µl). 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).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water.

Target Details

Target:	PROC
Alternative Name:	Protein C (PROC Products)
Target Type:	Viral Protein
Background:	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-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.

Application Details

Sample Volume:	25 µL
Assay Time:	< 3 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative competitive enzyme immunoassay technique that measures human Protein C in less than 3 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 competed with a biotinylated Protein C sandwiched by the immobilized antibody and 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.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. 2 Standard Curve: Reconstitute the 6 g of Protein C Standard with 1 ml of EIA Diluent to generate a solution of 6 g/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 (6 g/ml) 1:2 with equal volume EIA Diluent to produce 3, 1.5, 0.75, 0.375, 0.188 and 0.094 g/ml solutions. EIA Diluent serves as the zero standard (0 g/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Protein C] (g/ml) Standard (6 g/ml) P1 6.000 P2 1 part P1 + 1 part EIA Diluent 3.000 P3 1 part P2 + 1 part EIA Diluent 1.500 P4 1 part P3 + 1 part EIA Diluent 0.750 P5 1 part P4 + 1 part EIA Diluent 0.375 P6 1 part P5 + 1 part EIA Diluent 0.188 P7 1 part P6 + 1 part EIA Diluent 0.094 P8 EIA Diluent 0.000 Biotinylated Protein C (1x): Dilute Biotinylated Protein C with 4 ml EIA Diluent to produce a working solution. Allow to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): 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 EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes. Dilute samples 1:8 into EIA Diluent. 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). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:8 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Assay Procedure:	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 - 30 °C). 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. Add 25 µL of standard or sample per well, and immediately add 25 µL of Biotinlylated Protein C to each well (on top of the Standard or sample) and mix gently. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and

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	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. 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. Wash the microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 10 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. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. 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. 3
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 four-parameter or log-log logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.8% and 7.3% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- protein, 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. The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store SP Conjugate at -20°C Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a

Handling

	vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C. Store Standard and Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Publications

Product cited in:	Chan, Sy, Kong, Wong, Tse, Hon, Chan, Wong, Leung: "Childhood asthma is associated with polymorphic markers of PROC on 2q14 in addition to 17q21 locus." in: **Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology**, Vol. 26, Issue 2, pp. 173-80, (2015) (PubMed).