Datasheet for ABIN612669

APCS ELISA Kit

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Overview

Quantity:	96 tests
Target:	APCS
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.15 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Serum Amyloid P ELISA kit is designed for detection of human SAP in plasma, serum, urine, and cell culture supernatants
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	10% FBS in culture media will not affect the assay.
Components:	SAP Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human SAP. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. SAP Standard: Human SAP in a buffered protein base (40 ng, lyophilized). 1 Biotinylated SAP Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against SAP (140µl). MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A

Product Details

	20-fold concentrated buffered surfactant (30 ml, 2 bottles). 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:	APCS
Alternative Name:	Serum Amyloid P (APCS Products)
Background:	Amyloid P component, serum (SAP) is the identical serum form of amyloid P component (AP), a 25kDa pentameric protein. Serum amyloid P component is a normal plasma protein and a universal non-fibrillar constituent of amyloid deposits. Serum amyloid P (SAP) is a pentraxin similar to C-reactive protein. SAP and CRP are suggested to associate with cardiovascular disease. SAP is also suggested to link with Alzheimer's disease and liver disease.

Application Details

Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures SAP in less than 4 hours. A polyclonal antibody specific for SAP has been pre-coated onto a 96-well microplate with removable strips. SAP in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for SAP, 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.
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 up to 1 month at 2-8°C. 2 Standard Curve: Reconstitute the 40 ng of human SAP Standard with 4 ml of MIx Diluent to generate a stock solution of 10 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 stock solution (10 ng/ml) twofold with equal volume of MIx Diluent to produce 5, 2.5, 1.25, 0.625, 0.313, and 0.156 ng/ml. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [SAP] (ng/ml) P1 1 part Standard Stock (10 ng/ml) 10.00 P2 1 part P1 + 1 part MIx Diluent 5.000 P3 1 part P2 + 1 part MIx Diluent 2.500 P4 1 part P3 + 1 part MIx Diluent 1.250 P5 1 part P4 + 1 part MIx Diluent 0.625 P6 1 part P5 + 1 part MIx Diluent 0.313 P7 1 part P6 + 1 part MIx Diluent 0.156 P8 MIx Diluent 0.000 Biotinylated SAP Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIx Diluent. 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 MIx Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Plasma: Collect plasma using 3.8% sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:20000 into MIx Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA 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 1:20000 into MIx Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Collect cell culture media and centrifuge at 2000 x g for 10 minutes at 40C to remove debris. The samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes. Dilute urine samples 1:2 into MIx Diluent or within the range 1:2 to 1:20. Store samples 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. 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 50 µL of Standard or sample per well. 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 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 Biotinylated SAP Antibody to each well and incubate for one hour. Wash the microplate as described above. Add 50 µL of

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	Streptavidin-Peroxidase Conjugate per 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 approximately 15 minutes or till the optimal blue color density develop. Gently tap the 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.
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. 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 4.9 % and 7.1 % respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	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 and the biotinylated-antibody 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 and Biotinylated Antibody 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 vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with

Handling

	Diluent