Datasheet for ABIN1440223

SAA ELISA Kit

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Overview

Quantity:	96 tests
Target:	SAA
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.125-2 µg/mL
Minimum Detection Limit:	0.125 µg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human Serum Amyloid A ELISA (Enzyme-Linked Immunosorbent Assay) Ki t is designed for detection of SAA in human plasma, serum, and cell culture samples. This assay employs a quanti tative sandwich enzyme immunoassay technique that measures total SAA in approximately 4 hours . A polyclonal antibody speci fic for human SAA has been pre-coated onto a 96-well microplate with removable strips. SAA in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody speci fic for human SAA, which is recognized by a s treptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added . The color development is s topped and the intensi ty of the color is measured.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Plasma, Serum
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Cross-Reactivity (Details):	Cross-Reactivity: Canine < 1%, Monkey < 1%, Mouse < 2%, Swine < 5%. The conversion of IU and mg/mL is 1 International Unit (1IU) = 1.04 mg. Normal plasma SAA level is less than 10 µg/mL or 9.6 mU/mL. Standard has been calibrated against WHO reference standard
Components:	Human Serum Amyloid A Microplate: A 96-well polystyrene microplate (12 s trips of 8 wells) coated with a polyclonal antibody against human SAA. Sealing Tapes: Each ki t contains 3 precut, pressure sensi tive sealing tapes that can be cut to fi t the format of the individual assay. Human Serum Amyloid A Standard: Human SAA in a buffered protein base, calibrated against WHO 1 st International Standard (2 µg, 2 vials, lyophilized). Biotinylated Human Serum Amyloid A Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human SAA (120 l). MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Standard Diluent (1x): A buffered protein base with s tabilizer (2 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). SP Conjugate (100x): A 100-fold concentrate (80 l). Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution (1x): A 0.5 N hydrochloric acid solution to s top the chromogen substrate reaction (12 ml).
Material not included:	Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water. Incubator (37 °C)

Target Details

Target:	SAA
Abstract:	SAA Products
Background:	Human serum amyloid A (SAA) is a major apolipoprotein of high-density lipoprotein in plasma. It is not only synthesized by the liver and adipose tissue but also produced extrahepatically (1). SAA is a 12.5- kDa protein containing 122 amino acids with polymorphic forms (2-3). Four SAA genes have been identi fied and three encode functional proteins in humans . In response to inflammatory s timuli , acute-phase SAA1 and SAA2 are secreted and increased . SAA3 is a pseudogene that does not express protein . SAA4 is expressed consti tutively in the liver (4).
Gene ID:	62886289

Application Details

Sample Volume:	50 µL

Application Details

Assay Time:	4 h
Plate:	Pre-coated
Protocol:	• Step 1. Add 50 µL of Standard or Sample per well. Incubate 2 hours. • Step 2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour. • Step 3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes. • Step 4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 15 minutes. • Step 5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.
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 Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8 °C. Human Serum Amyloid A Standard: Reconsti tute the Human Serum Amyloid A Standard (2 µg) with 0.5 mL of Standard Diluent to generate a 4 µg/mL standard s tock solution. Allow the vial to sit for 10 minutes with gentle agi tation prior to making dilutions. Prepare duplicate or triplicate s tandard points by serially diluting from the standard stock solution (4 µg/mL) 2-fold with equal volume of MIX Diluent to produce 2, 1, 0.5, 0.25, and 0.125 µg/mL solutions. MIX Diluent serves as the zero s tandard (0 µg/mL). Aliquot remaining s tock solution to limit repeated freeze-thaw cycles . This solution should be s tored at -20 °C and used within 48 hours. Standard Point Dilution [SAA] (µg/mL) [SAA] (mIU/mL) P1 1 part Standard + 1 part MIX Diluent 2.0 1.92 P2 1 part P1 + 1 part MIX Diluent 1.0 0.96 P3 1 part P2 + 1 part MIX Diluent 0.5 0.48 P4 1 part P3 + 1 part MIX Diluent 0.25 0.24 P5 1 part P4 + 1 part MIX Diluent 0.125 0.12 P6 MIX Diluent 0.0 0.0 Biotinylated Human Serum Amyloid A Antibody (50x): Spin down the antibody briefly and dilute the desi red amount of the antibody 50-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be s tored 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 20-fold with reagent grade water to produce a 1x solution. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desi red amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be s tore d at -20 °C. 5
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma . A 4-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs . The undiluted samples can be s tored 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). Serum: Samples should be collected into a serum separator tube . After clot formation, centri fuge samples at 3000 x g for 10 minutes and remove serum. A 4-fold sample

	dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. 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 centri fuge at 3000 x g for 10 minutes at 4 °C to remove debris and collect supernatants . Samples can be s tored at -20 °C or below. Avoid repeated freeze-thaw cycles .
Assay Procedure:	Prepare all reagents , standard solutions , and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove excess microplate s trips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and s tore in a vacuum desiccator. Add 50 l of Human Serum Amyloid A Standard or sample 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 2 hours . Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents , hi t 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents , hi t 4-5 times on absorbent material to completely remove the liquid. Add 50 l of Biotinylated Human Serum Amyloid A Antibody 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 1 hour. Wash the microplate as described above . Add 50 l of 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. Wash the microplate as described above . Add 50 l of Chromogen Substrate to each well . Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 15 minutes or until the optimal blue color density develops. Add 50 l 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. 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 a t high concentration points after s topping the reaction for about 10 minutes, which will reduce the readings . 6
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 (OD) on the y-axis. The best-fit line can be

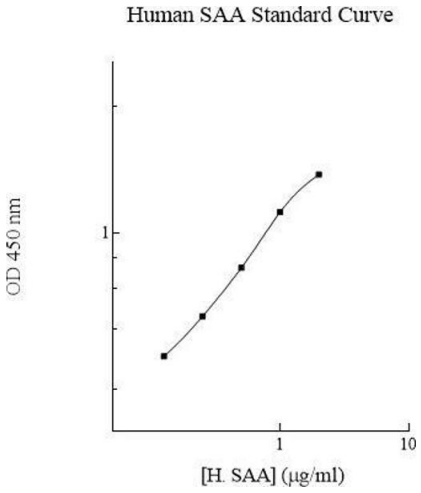
Application Details

	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.
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:	This product is for Research Use Only and is not intended for use in diagnostic procedures . Prepare all reagents (diluent buffer, wash buffer, s tandard, 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 insert. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial , the biotinylated antibody vial , and the s tandard diluent vial before opening and using contents. The Stop Solution is an acidic solution. The ki t should not be used beyond the expiration date. 2
Storage:	4 °C/-20 °C
Storage Comment:	Upon arrival , immediately store components of the ki t at recommended temperatures up to the expiration date. Store Standard, SP Conjugate , and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Standard Diluent (1x), 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 s tored for up to 30 days in a vacuum desiccator.

Images



ELISA

Image 1.