Datasheet for ABIN456394

SAA1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	SAA1
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of human Serum amyloid A, SAA concentrations in cell culture supernates, serum, plasma and other biological fluids.
Sample Type:	Cell Culture Supernatant, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural human SAA.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Characteristics:	Homo sapiens,Human,Serum amyloid A-1 protein,SAA,SAA1
Components:	Reagent (Quantity): • Assay plate (1), • Standard (2), • Sample Diluent (1×20 mL), • Assay Diluent A (1×10 mL),

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	• Assay Diluent B (1×10 mL), • Detection Reagent A (1×120 µL), • Detection Reagent B (1×120 µL), • Wash Buffer(25 x concentrate) (1×30 mL), • Substrate (1×10 mL), • 2 Stop Solution (1×10 mL), • Plate sealer for 96 wells (5), • Instruction (1)
Material not included:	Microplate reader. Pipettes and pipette tips. EP tube Deionized or distilled water.

Target Details

Target:	SAA1
Alternative Name:	SAA1 (SAA1 Products)
Background:	Serum amyloid A (SAA) proteins are a family of apolipoproteins found predominantly associated with high-density lipoprotein (HDL) in plasma, with different isoforms being unequally expressed constitutively and in response to inflammatory stimuli. Although synthesized primarily in the liver, extrahepatic tissue_cellular expression of SAA has been widely documented. SAA has been linked to functions related to inflammation, pathogen defense, HDL metabolism, and cholesterol transport and thereby has been implicated in several pathological conditions including atherosclerosis, rheumatoid arthritis, Alzheimer's disease, and cancer. SAA is known best for its role during the acute phase response to an inflammatory stimulus such as infection, tissue injury, and trauma. During active inflammation the concentration of SAA in plasma can increase up to 1,000-fold within 24 h. It is believed that persistently high levels of SAA during chronic inflammation may contribute to the occasional development of the potentially fatal disease reactive amyloidosis (amyloid A (AA) amyloidosis). In AA amyloidosis, AA, an N-terminal (1-76) fragment of SAA, frequently is found to form amyloid deposits in the liver, kidney, and spleen. However, the presence, in vivo, of full-length SAA in amyloid deposits and the ability of various SAA isoforms to form fibrils in vitro suggest that proteolytic cleavage may not be a prerequisite for AA deposition but rather a postdeposition event. There is very limited structural information about SAA because of its inherent poor solubility in the apolipoprotein form. It is intriguing to understand how such a small protein is able to mediate or directly carry out such a wide range of functions related to inflammatory reaction and other hostdefense mechanisms. The various functions of SAA may be modulated by factors such as conformational changes induced by ligand binding or by the ability to adopt more than one oligomeric state. Deciphering the molecular basis of the functional and potentially pathological

Target Details

	properties of SAA will require understanding its structure under various conditions.
Pathways:	Toll-Like Receptors Cascades

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to SAA. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for SAA and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain SAA, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of SAA in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard. The Sample Diluent serves as the zero standard (0 ng/ml).
Sample Collection:	Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20 °C or -80° C . Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g at 2 - 8 C within 30 minutes of collection. Store samples at -20 °C or -80 °C . Avoid repeated freeze-thaw cycles. Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 °C or -80 °C . Avoid repeated freeze-thaw cycles. Note: Serum, plasma, and cell culture supernatant samples to be used within 7 days may be

	stored at 2-8 °C , otherwise samples must stored at -20 °C (≤ 1 months) or -80 °C (≤ 2 3 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature. Sample preparation Serum/plasma samples require a 100 fold dilution. A suggested 100-fold dilution is 10 uL sample + 990 uL Sample Diluent.
Assay Procedure:	Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 °C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 °C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. 1. Add 100 µL of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37 °C . 2. Remove the liquid of each well, don ' t wash. 3. Add 100 µL of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 °C . Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform. 4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 5. Add 100 µL of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37 °C . 6. Repeat the aspiration/wash as in step 4. 7. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37 °C . Protect from light. 8. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 9. Determine the optical density of each well at once, using a microplate reader set to 450 nm. Important Note: 1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-

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	wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption. 2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 μ l for once pipetting. 3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the 5 strips DRY at any time during the assay. 4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes. 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 7. Duplication of all standards and specimens, although not required, is recommended. 8. Substrate Solution is easily contaminated. Please protect it from light.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the SAA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label. 2. Do not mix or substitute reagents with those from other lots or sources.

Handling

	3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. 4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	The Assay Plate, Standard, Detection Reagent A and Detection Reagent B should be stored at -20°C upon being received. After receiving the kit , Substrate should be always stored at 4°C.