Innovative RESEARCH Results Driven Research

Human Serum Amyloid A (SAA) ELISA Kit

Introduction

Human Serum Amyloid A (SAA) is a major apolipoprotein of high-density lipoprotein in plasma and a sensitive marker of acute inflammation. It is not only synthesized by the liver and adipose tissue, but also produced extrahepatically by many cancers (1). SAA is a 12.5-kDa protein containing 122 amino acids with polymorphic forms (2-3). Four SAA genes have been identified and three encode functional proteins in human. In response to inflammatory stimuli, acute-phase SAA1 and SAA2 are secreted and increased. Whereas SAA3 is a pseudogene that does not express protein, and SAA4 is expressed constitutively in the liver (4). SAA is associated with obesity, amyloidosis, type 2 diabetes, atherosclerosis, metabolic syndrome, rheumatoid arthritis, and renal and lung cancers (5-9).

Principal of the Assay

The Human Serum Amyloid A ELISA kit is designed for detection of human SAA in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures total SAA in less than 4 hours. A monoclonal antibody specific for 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 the biotinylated polyclonal antibody specific for SAA, 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.

Caution and Warning

- **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.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **SAA Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human SAA.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **SAA Standard:** Human SAA in a buffered protein base (0.6 µg, lyophilized, 1 vial) (Calibrated against WHO 1st International Standard).
- **Biotinylated SAA Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against SAA (140 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, 2 bottles).
- **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 Condition

- Store components of the kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Store Standard, SP Conjugate and Biotinylated Antibody at -20^{0}C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8^{0}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^{0}C.
- Store Standard at -20^{0}C before reconstituting with Diluent and after reconstituting with Diluent.

Other Supplies Required

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

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using 3.8% sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes. Dilute samples 1:20 into MIX Diluent and assay. If necessary dilute samples within the range of 1:10 to 1:200. The undiluted samples can be stored at -20^{0}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 dilute samples 1:20 into MIX Diluent. If necessary dilute samples within the range of 1:10 to 1:200. The undiluted samples can be stored at -20^{0}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 4^0C to remove debris. The samples can be stored at -20^0C or below. Avoid repeated freeze-thaw cycles.

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 1 month at 2-8^0C.
- **Standard Curve:** Reconstitute the 0.6 µg of human SAA Standard with1.2 ml of MIX Diluent to generate a stock solution of 0.5 µg/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 (0.5 µg/ml) 1:2 with equal volume of MIX Diluent to produce 0.25, 0.125, 0.0625, 0.0313, 0.0156 and 0.0078 µg/ml. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C and used within the next 30 days.

Standard Point	Dilution	[SAA] (µg/ml)	[SAA] (mU/ml)
P1	1 part Standard (0.5 µg/ml)	0.500	0.48
P2	1 part P1 + 1 part MIX Diluent	0.250	0.24
P3	1 part P2 + 1 part MIX Diluent	0.125	0.12
P4	1 part P3 + 1 part MIX Diluent	0.0625	0.06
P5	1 part P4 + 1 part MIX Diluent	0.0313	0.03
P6	1 part P5 + 1 part MIX Diluent	0.0156	0.015
P7	1 part P6 + 1 part MIX Diluent	0.0078	0.0075
P8	MIX Diluent	0.0000	0.0000

- **Biotinylated SAA 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 -20^0C.
- **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 -20^0C.

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 SAA Antibody to each well and incubate for one hour.

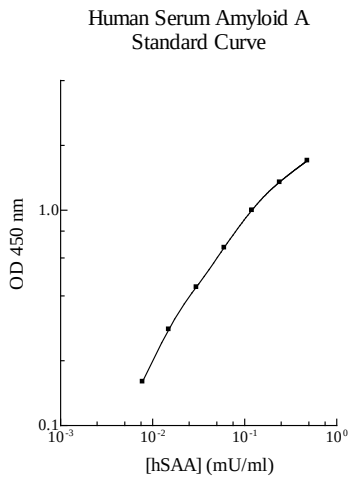
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for approximately 20 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.

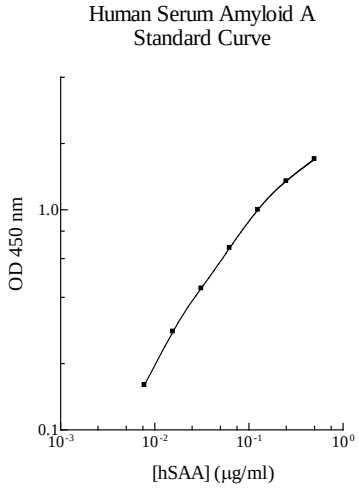
Data Analysis

- 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 used for illustration only. A standard curve should be generated each time the assay is performed.





Performance Characteristics

- The minimum detectable dose of human SAA is typically ~0.007 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.1 % respectively.
- Standard has been calibrated against WHO reference standard

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:10	85%	84%
1:20	98%	97%
1:40	105%	103%

Recovery

Standard Added Value	0.02 – 0.2 µg/ml
Recovery %	80-112 %
Average Recovery %	96%

Cross-Reactivity

Species	% Cross Reactivity
Canine	< 1%
Bovine	None
Monkey	< 1%
Mouse	< 2%
Rat	None
Swine	< 5%
Rabbit	None

Note:

- **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.

References

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Version 1.8