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Human SAA4 Immunoassay

Catalog Number: SEKH-0386

For the quantitative determination of human SAA4 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of SAA4 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	102	101
	Range (%)	91-112	93-108
1:4	Average% of Expected	96	103
	Range (%)	87-107	95-112
1:8	Average% of Expected	93	104
	Range (%)	85-103	93-114
1:16	Average% of Expected	95	106
	Range (%)	84-103	98-117

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 320pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human SAA4. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

BMP1, BMP2, BMP4, BMP7, CRP, CCL2, CCL4, CCL5, HGF, HSP27, IGF-1, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-15, IL-17C, IL-21, IFNγ, PDGF, PLA2G7, serpin E1, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TLR9, TNF-α, TNF RI, TNF RII, VEGF.

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of SAA4 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of SAA4 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	93	89-96
Cell culture supernatants	107	99-114

BACKGROUND

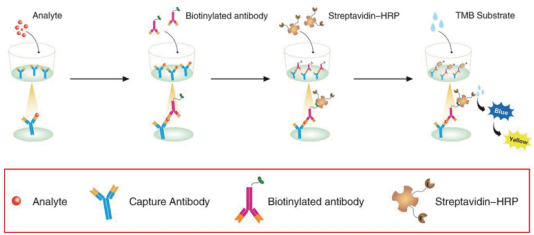
Serum amyloid A (SAA) proteins are a family of apolipoproteins associated with high-density lipoprotein (HDL) in plasma. Different isoforms of SAA are expressed constitutively (constitutive SAAs) at different levels or in response to inflammatory stimuli (acute phase SAAs). These proteins are produced predominantly by the liver. Acute-phase SAA proteins (A-SAAs) are secreted during the acute phase of inflammation. These proteins have several roles, including the transport of cholesterol to the liver for secretion into the bile, the recruitment of immune cells to inflammatory sites, and the induction of enzymes that degrade extracellular matrix. A-SAAs are implicated in several chronic inflammatory diseases, such as amyloidosis, atherosclerosis, and rheumatoid arthritis. Three acute-phase SAA isoforms have been reported in mice, called SAA1, SAA2, and SAA3. During inflammation, SAA1 and SAA2 are expressed and induced principally in the liver, whereas SAA3 is induced in many distinct tissues. SAA1 and SAA2 genes are regulated in liver cells by the proinflammatory cytokines IL-1, IL-6, and TNF-α. Both SAA1 and SAA2 are induced up to a 1000-fold in mice under acute inflammatory conditions following exposure to bacterial lipopolysaccharide. SAA is also an acute phase marker. Similar to CRP, levels of acute-phase SAA increase within hours after inflammatory stimulus, and the magnitude of increase may be greater than that of CRP. Relatively trivial inflammatory stimuli can lead to SAA responses. It has been suggested that SAA levels correlate better with disease activity in early inflammatory joint disease than do ESR and CRP. Although largely produced by hepatocytes, SAA is produced by adipocytes as well, and its serum concentration is associated with body mass index. A fourth SAA (SAA4) was identified in humans and is expressed constitutively in the liver and, thus, is defined as a constitutive SAA (C-SAA). The originally designated SAA5 in the mouse is now called SAA4.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for SAA4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SAA4 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for SAA4

is added to detect the captured SAA4 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



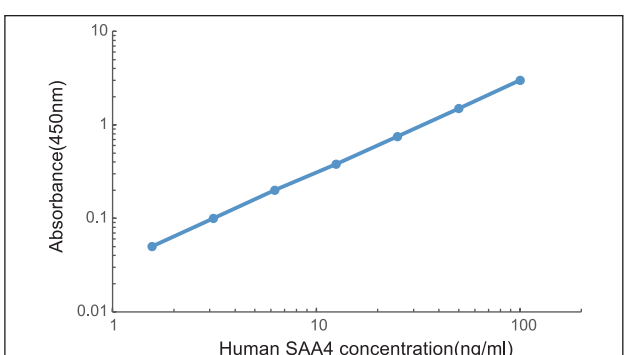
TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

4. The data may be linearized by plotting the log of the SAA4 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. 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.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the SAA4 ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0	0.034	0.036	0.035	---
1.56	0.072	0.077	0.075	0.040
3.125	0.101	0.106	0.104	0.069
6.25	0.176	0.169	0.173	0.138
12.5	0.344	0.326	0.335	0.300
25	0.592	0.554	0.573	0.538
50	1.293	1.078	1.186	1.151
100	2.187	2.259	2.223	2.188



Representative standard curve for SAA4 ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-human SAA4 antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 30 minutes at room temperature (25±2℃).

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 5-30 minutes (depending on signal),at room temperature(25±2℃).Protect from light.

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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 y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized, 100 ng/ml upon reconstitution	1 vial	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody (100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 bottle	Store at 2-8°C **for six months
Standard /Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

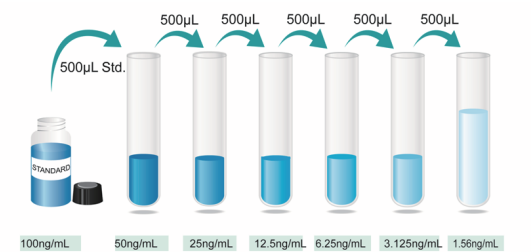
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note:It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Sample** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 100ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500μL of Standard/Sample Diluent into 50ng/ml tube and the remaining tubes. Use the stock solution of 100ng/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 100ng/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of SAA4 standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-human SAA4 antibody**: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**