Human Serum Amyloid A (SAA) ELISA

Cat. No.: BA1006

Enzyme Immunoassay for the quantitative determination of Serum amyloid A (SAA) in human serum, plasma, and other body fluids.
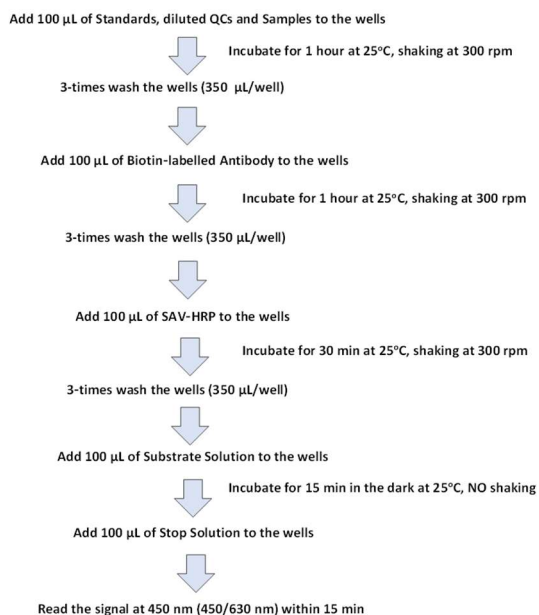
Serum amyloid A (SAA) is a highly sensitive acute phase reactant that has been linked to inflammatory diseases, both infectious and non-infectious origin. SAA is an apolipoprotein that interacts with HDL, promotes the accumulation of leukocytes at the site of inflammation and the adhesion of platelets, and participates in the removal of damaged cell membranes. The acute phase SAAs (SAA1, SAA2) are transcriptionally regulated in hepatocytes by a variety of inflammatory cytokines, and they can transiently increase > 1000-fold. The combination of SAA, PCT, and CRP can improve the differential diagnosis of early bacterial and viral infections. Several studies indicated that SAA allows monitoring of disease activity in various inflammatory rheumatic diseases and autoinflammatory diseases, including SLE, amyloidosis, or rheumatoid arthritis. SAA can be also used to risk prediction of coronary heart disease, the prognosis of tumour patients, and observation of transplant rejection.

PRINCIPLE OF SAA ELISA

The microtiter plate is coated with the antibody specifically binding the Serum amyloid A (SAA1 and SAA2). The human serum or plasma is incubated in the plate with the capture antibody.
The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.
Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).
The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of SAA in the specimen. The concentration of SAA in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.

Add 100 μL of Standards, diluted QCs and Samples to the wells

Incubate for 1 hour at 25°C, shaking at 300 rpm

3-times wash the wells (350 μL/well)

Add 100 μL of Biotin-labelled Antibody to the wells

Incubate for 1 hour at 25°C, shaking at 300 rpm

3-times wash the wells (350 μL/well)

Add 100 μL of SAV-HRP to the wells

Incubate for 30 min at 25°C, shaking at 300 rpm

3-times wash the wells (350 μL/well)

Add 100 μL of Substrate Solution to the wells

Incubate for 15 min in the dark at 25°C, NO shaking

Add 100 μL of Stop Solution to the wells

Read the signal at 450 nm (450/630 nm) within 15 min

Kit Contents

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody 100x conc.	140 μL
Ab-bt diluent	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer 2x conc.	2x13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

Preparation of Standards

Reconstitute lyophilized Human SAA Master Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human SAA in Master Standard is 200 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard to produce a dilution series (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 200 ng/mL (lyophilised)	See CofA	200 ng/mL
Std2	300 μL of Std1	300 μL	100 ng/mL
Std3	300 μL of Std2	300 μL	50 ng/mL
Std4	300 μL of Std3	300 μL	25 ng/mL
Std5	300 μL of Std4	300 μL	12.5 ng/mL
Std6	300 μL of Std5	300 μL	6.25 ng/mL
Blank	-	300 μL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls in deionized/distilled, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:50 in Dilution Buffer, prior to use, see Preparation of samples.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH$_2$O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of Dilution Buffer 1x

Prepare a working solution of Dilution Buffer by mixing 13 mL (26 mL) of Dilution Buffer 2x conc. and 13 mL (26 mL) of deionized/ distilled water (dH$_2$O). Prepare only the amount for immediate consumption. Mix well. Store at 4°C for two weeks.

Preparation of Biotin-Labelled Antibody 1x

Prepare a working solution of Biotin-labelled Antibody by mixing 130 µL of Biotin-labelled Antibody 100x conc. and 12.870 mL of Ab-bt Diluent in the special tube. Mix well. Store at 4°C for two weeks.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of healthy individual samples is 1:50, i.e., 5 µL of sample + 245 µL of Dilution Buffer, for duplicates and for singlets.

Recommended dilution of samples is 1: 2000 for individuals in condition in which is expected higher level of SAA5. It is recommended to use the two-step dilution.
Dilution A (40x) for both singlets and duplicates: 5 µL of samples + 195 µL of Dilution Buffer.
Dilution B (50x): 5 µL of Dilution A + 245 µL of Dilution Buffer, for singlets; 15 µL of Dilution A + 285 µL of Dilution Buffer, for duplicates.
Recommended dilution of **urine** is 1:10, i.e., 20 of samples + 180 µL of Dilution Buffer, for singlets, 30 of samples + 270 µL of Dilution Buffer, for duplicates.
Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ± 2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for **1 hour** at 25°C ± 2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ± 2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for **15 min,** at 25°C ± 2°C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450/630 nm within 15 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:50 as recommended and assayed. The results are multiplied by dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human SAA giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 1.56 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (µg/ml)	SD	CV (%)
1	15.0	1.4	9
2	0.9	0.1	10

Inter-assay (Run – to – run)

Sample	Mean (µg/ml)	SD	CV (%)
1	4.0	0.2	4
2	1.2	0.04	3

3. Accuracy

Dilution linearity

Sample	Dilution	Measured concentration (µg/ml)	Expected concentration (µg/ml)	Yield (%)
1		4.6	-	-
	2x	2.4	2.3	103
	4x	1.4	1.1	119
	8x	0.7	0.6	117
2		10.8	-	-
	2x	5.3	5.4	98
	4x	2.8	2.7	104
	8x	1.6	1.4	120

Spiking Recovery

Sample	Spike (ng /mL)	Measured concentration (µg/ml)	Expected concentration (µg/ml)	Yield (%)
1	-	1.1	-	-
	2.5	3.2	3.6	89
	1.3	2.2	2.3	94
	0.6	1.7	1.7	102

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.

BioLab Assays

Human SAA Standard Curve
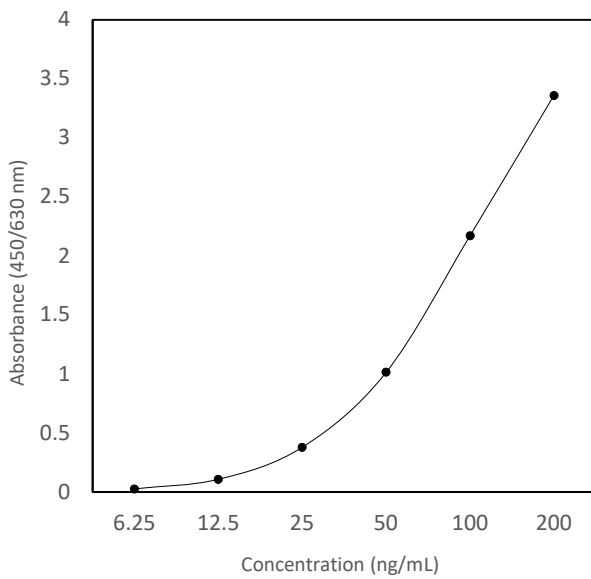
RESOURCES

[1]Levin M, Franklin EC, Frangione B, Pras M. The amino acid sequence of a major nonimmunoglobulin component of some amyloid fibrils. J Clin Invest. 1972;51:2773–6. https://doi.org/10.1172/ JCI107098. 3. Linke RP, Sipe JD, Pollock PS, Ignaczak TF, Glenner GG. Isolation of a low-molecular-weight serum component antigenically related to an amyloid fibril protein of unknown origin. Proc Natl Acad Sci U S A. 1975;72:1473–6. https://doi.org/10.1073/pnas.72. 4.1473.

[2]Johnson BD, Kip KE, Marroquin OC, Ridker PM, Kelsey SF, Shaw LJ, et al. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). Circulation. 2004;109:726–32. https://doi.org/10.1161/01.CIR.0000115516.54550.B1. 19. Kosuge M, Ebina T, Ishikawa T, Hibi K, Tsukahara K, Okuda J, et al. Serum amyloid A is a better predictor of clinical outcomes than C-reactive protein in non-ST-segment elevation acute coronary syndromes. Circ J. 2007;71:186–90. https://doi.org/10.1253/circj. 71.186. 20. Deguchi H, Elias DJ, Navarro S, Espana F, Griffin JH. Elevated serum amyloid A is associated with venous thromboembolism. Thromb Haemost. 2013;109:358–9. https://doi.org/10.1160/ TH12-10-0722.

[3]Saiki O, Uda H. Ratio of serum amyloid A to C-reactive protein is constant in the same patients but differs greatly between patients with inflammatory diseases. Scand J Immunol. 2022 Feb;95(2):e13121. doi: 10.1111/sji.13121. Epub 2021 Nov 24. PMID: 34796986.