Human SAA ELISA Kit

Catalog Number KHA0011 (96 tests), KHA0012 (2 × 96 tests), KHA0011C (5 × 96 tests)

Pub. No. MAN0003952 **Rev.** 6.0 (32)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human SAA ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human SAA in serum, plasma, buffered solutions, or tissue culture supernatants. The assay will recognize both natural and recombinant human SAA.

SAA proteins are involved in acute phase responses to inflammation. They are released into the bloodstream upon synthesis where they immediately bind to HDL. During the acute phase, circulating SAA levels are increased by 100–1000 fold, reaching concentrations ≤1 mg/mL.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHA0011 (96 tests)
Hu SAA Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.5% Proclin™ 300	100 mL
Hu SAA Antibody Coated Plate, 96-well strip-well plate	1 plate
Hu SAA Biotin Conjugate; contains 0.1% sodium azide	6 mL
Streptavidin-HRP (100X)	0.125 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

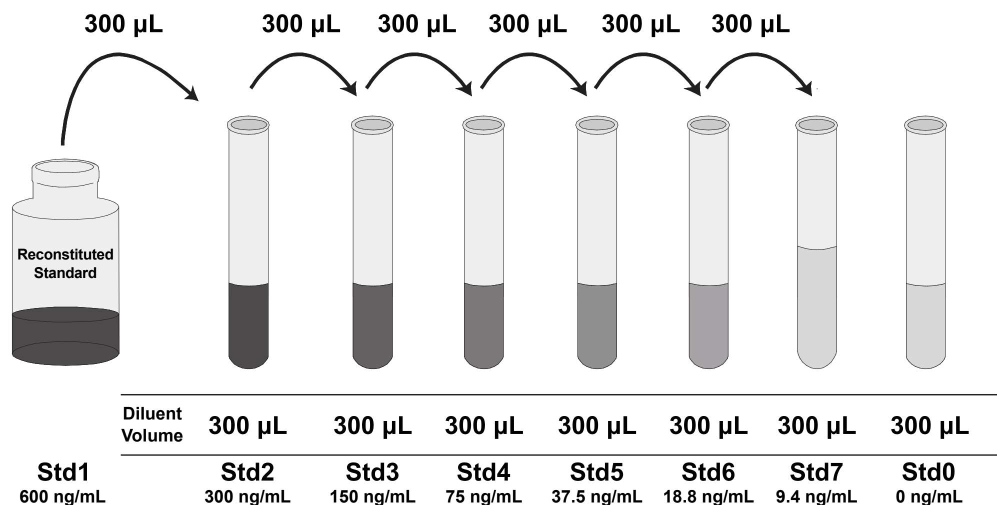
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Analyze **tissue culture supernatant** samples without dilution.
- Perform sample dilutions with Standard Diluent Buffer.
- Dilute **serum** and **plasma** samples 200-fold in Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu SAA Standard to 600 ng/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 600 ng/mL human SAA. Use the standard within 15 minutes of reconstitution.
2. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 300, 150, 75, 37.5, 18.8, 9.4, and 0 ng/mL human SAA.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 3 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen

a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Add 50 µL Hu SAA Biotin Conjugate solution into each well except the chromogen blanks.
c. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

3 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

4 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to600 ng/mL human SAA.

Standard Human SAA (ng/mL)	Optical Density (450 nm)
600	3.34
300	2.33
150	1.31
75	0.63
37.5	0.38
18.8	0.20
9.4	0.15
0	0.06

High-dose hook effect

No hook effect was observed with concentrations up to 50 µg/mL.

Inter-assay precision

Samples were assayed 36 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	61.3	198.4	598.8
Standard Deviation	4.8	14.7	42.0
% Coefficient of Variation	7.8	7.4	7.0

Intra-assay precision

Samples of known human SAA concentration were assayed in replicates of 12 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	61.7	203.6	585.8
Standard Deviation	4.6	9.3	36.6
% Coefficient of Variation	7.4	4.6	6.2

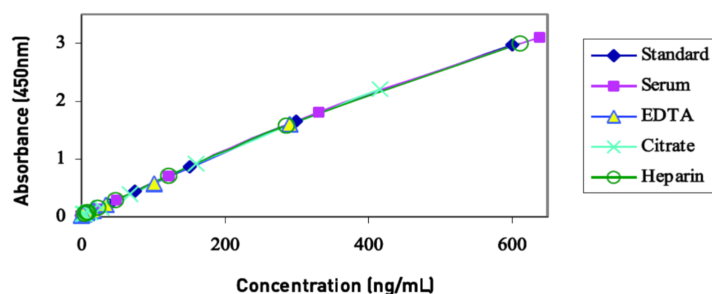
Linearity of dilution

Human serum, EDTA plasma, citrate plasma, heparin plasma, and tissue culture medium spiked with recombinant human SAA were serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded the following correlation coefficients:

Sample	Correlation Coefficient
Serum	1.00
EDTA plasma	0.97
Citrate plasma	1.00
Heparin plasma	1.00
Tissue culture supenatant	1.00

Parallelism

Random human serum, EDTA plasma, citrate plasma, and heparin plasma samples were serially diluted in the Standard Diluent Buffer and analyzed. The optical density of each dilution was plotted against the human SAA standard curve.



Recovery

The recovery of recombinant human SAA added to human serum, EDTA plasma, citrate plasma, heparin plasma, and tissue culture medium containing 10% fetal bovine serum or 10% calf serum was measured with the Human SAA ELISA Kit.

Sample	Average % Recovery
Serum [1]	114
EDTA plasma [1]	111
Citrate plasma [1]	108
Heparin plasma [1]	84
RPMI + 10% fetal bovine serum	102
DMEM + 10% calf serum	89

[1] Samples were pre-diluted 200-fold in the Standard Diluent Buffer.

Sensitivity

The analytical sensitivity of this assay is <4 ng/mL human SAA. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

Specificity

Buffered solutions of a panel of substances ranging in concentration from 5 to10 μg/mL were assayed with the Human SAA ELISA Kit and found to have no cross-reactivity: **human** CRP, IL-2, haptoglobin, IL-1, IL-3, IL-6, IL-7, IL-13, PDGF-BB, GRO-, IFN-γ, SCF, TNF-, VEGF; **mouse** CRP, IL-1, IL-13, KC, SCF, eotaxin, TNF-, VEGF, SAA1, SAA2; **rat** CRP, IL-1, IL-2, GRO, VEGF.

Limited product warranty

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