Human SAA ELISA Kit

Catalog. nos. KHA0011
KHA0012
KHA0011C

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Description

The Human Serum Amyloid A (Hu 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 the acute phase responses to inflammation. They are released into the bloodstream upon synthesis where they immediately bind to HDL particles. During the acute phase, circulating SAA levels are increased by 100–1000 fold, reaching concentrations ≤ 1 mg/mL.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	Cat. no. KHA0011 96 Test Kit	Cat. no. KHA0012 192 Test Kit	Cat. no. KHA0011C 480 Test Kit
Hu SAA Antibody Coated Wells. 96 well plate.	1 plate	2 plates	5 plates
Hu SAA Biotin Conjugate. Contains 0.1% sodium azide.	6 mL	2 × 6 mL	5 × 6 mL
Hu SAA Standard. Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials	10 vials
Wash Buffer Concentrate (25X).	100 mL	100 mL	2 × 100 mL
Standard Diluent Buffer. Contains 0.5% ProClin™ 300.	100 mL	2 × 100 mL	5 × 100 mL
Streptavidin-HRP (100X). Contains 3.3 mM thymol.	0.125 mL	2 × 0.125 mL	5 × 0.125 mL
Streptavidin-HRP Diluent. Contains 3.3 mM thymol.	25 mL	25 mL	3 × 25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL	25 mL	3 × 25 mL
Stop Solution.	25 mL	25 mL	3 × 25 mL
Adhesive Plate Covers.	3	6	15

CAUTION! This kit contains materials with small quantities of sodium azide and ProClin 300. 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. ProClin 300 is toxic. 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.

Materials required but not provided

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

General guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com** for details before starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- 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.

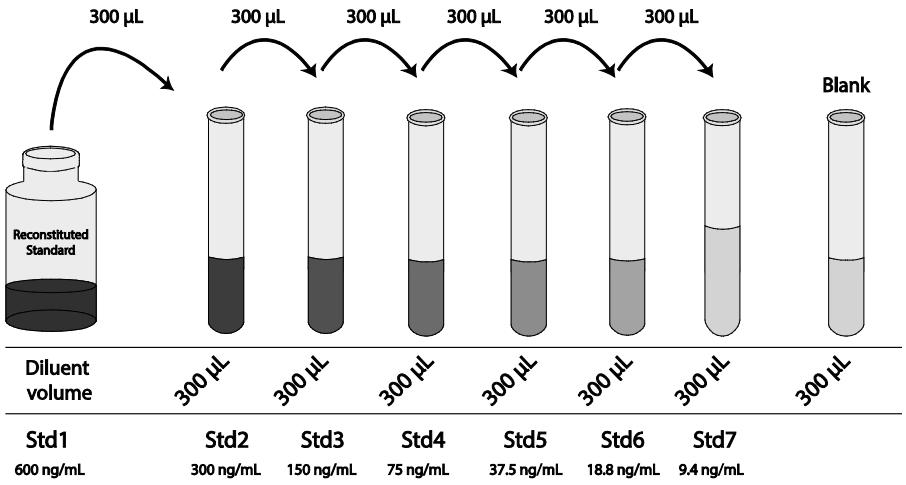
Dilute samples

- Dilute **serum and plasma** samples 200-fold in Standard Diluent Buffer.
- **Tissue culture supernatant** samples can be analyzed without dilution.
- Samples that are >600 ng/mL should be diluted with Standard Diluent Buffer
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

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 Hu SAA.
   Use the standard within 15 minutes of reconstitution. The remaining reconstituted standard can be stored in aliquots at –80°C, and can be frozen and thawed one time without loss of immunoreactivity.
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 Hu SAA.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

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

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. Allow Streptavidin-HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
3. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

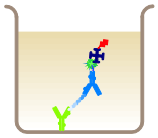
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3 hours.**

IMPORTANT! Perform a standard curve with each assay.

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 to 8°C for future use.

Bind antigen

1. Add 100 µL of standards and diluted or undiluted samples (see page 2) to the appropriate wells.
2. Add 50 µL Hu SAA Biotin Conjugate solution into each well except chromogen blanks.
3. Cover the plate with plate cover and incubate for 2 hours at room temperature.
4. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer.



Add Streptavidin-HRP

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

Add chromogen

8. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
9. Cover the plate with plate cover and incubate for 30 minutes at room temperature **in the dark**.

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

Add stop solution

10. Add 100 µL Stop Solution to each well. Tap side of the plate gently 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 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four 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 that of the highest standard 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 0–600 ng/mL Hu SAA.

Standard Hu SAA (ng/mL)	Optical Density (450nm)
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

Specificity

Buffered solutions of a panel of substances ranging in concentration from 5–10 µg/mL were assayed with the Hu SAA kit and found to have no cross-reactivity: **Human** CRP, IL-2, haptoglobin, IL-1α, IL-3, IL-6, IL-7, IL-13, PDGF-BB, GRO-α, IFN-γ, SCF, TNF-α, VEGF; **Mouse** CRP, IL-1α, IL-13, KC, SCF, eotaxin, TNF-α, VEGF, SAA1, SAA2; **Rat** CRP, IL-1α, IL-2, GRO, VEGF.

Sensitivity

The minimum detectable dose of Hu SAA is <4 ng/mL. 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.

Performance characteristics, continued

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	61.7	203.6	585.8
SD	4.6	9.3	36.6
%CV	7.4	4.6	6.2

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	61.3	198.4	598.8
SD	4.8	14.7	42.0
%CV	7.8	7.4	7.0

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recovery of recombinant Hu 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 Hu SAA ELISA Kit.

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

* Samples were pre-diluted 200-fold in the Standard Diluent Buffer.

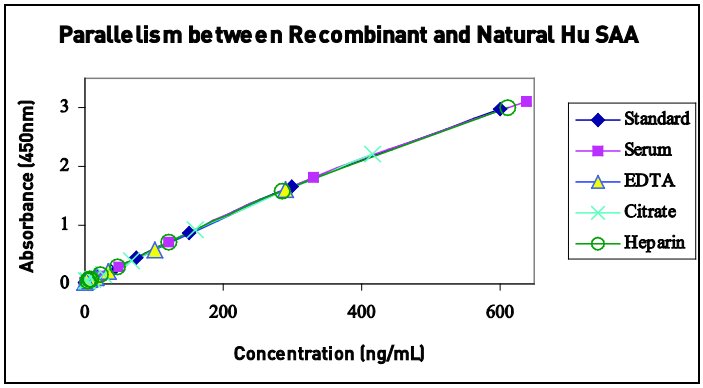
Linearity of dilution

Human serum, EDTA plasma, citrate plasma, heparin plasma, and tissue culture medium spiked with recombinant Hu 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
Serum	0.996
EDTA plasma	0.973
Citrate plasma	0.995
Heparin plasma	0.997
Tissue culture supenatant	0.999

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 Hu SAA standard curve. Parallelism is demonstrated in the following figure.



High dose hook effect

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

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

For support visit thermofisher.com/support or contact **techsupport@lifetech.com**.

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