AssayMax™

Human Serum AA ELISA Kit

Assaypro LLC
3400 Harry S Truman Blvd
St. Charles, MO 63301
T (636) 447-9175
F (636) 395-7419
www.assaypro.com

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 15 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Human Serum Amyloid A ELISA Kit

Catalog No. EA8001-1

Sample insert for reference use only

Introduction

Human serum amyloid A (SAA) is a major apolipoprotein of high-density lipoprotein in plasma. It is not only synthesized by the liver and adipose tissue, but also produced extrahepatically (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 humans. In response to inflammatory stimuli, acute-phase SAA1 and SAA2 are secreted and increased. SAA3 is a pseudogene that does not express protein, and SAA4 is expressed constitutively in the liver (4).

Principle of the Assay

The AssayMax Human Serum Amyloid A ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human SAA in **plasma, serum, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures total SAA in less than 4 hours. A polyclonal 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 antibody specific for SAA, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is 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

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, 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 insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial, the biotinylated antibody vial, and the standard vial before opening and using contents.
- The Stop Solution is an acidic solution.

- The kit should not be used beyond the expiration date.

Reagents

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

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, SP Conjugate, and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.

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 one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:4 into MIX Diluent, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:4 into MIX Diluent, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3000 x *g* for 10 minutes at 4°C to remove debris. Samples can be stored at -20°C 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 Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Briefly spin down the standard vial before opening and using contents. **Aliquot standard to limit repeated freezing and thawing.** Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (12 µg/ml) 1:6 for the first point (2 µg/ml), then 1:2 with equal volume of MIX Diluent to produce 1, 0.5, 0.25, and 0.125 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution in the aliquot tube should be frozen at -20°C and used within 2 days. **Avoid repeated freeze-thaw cycles.**

Standard Point	Dilution	[SAA] (µg/ml)	[SAA] (mU/ml)
P1	1 part Standard (12 µg/ml) + 5 parts MIX Diluent	2.000	1.920
P2	1 part P1 + 1 part MIX Diluent	1.000	0.960
P3	1 part P2 + 1 part MIX Diluent	0.500	0.480
P4	1 part P3 + 1 part MIX Diluent	0.250	0.240
P5	1 part P4 + 1 part MIX Diluent	0.125	0.120
P6	MIX Diluent	0.000	0.000

- **Biotinylated Human Serum AA 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°C.
- **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°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human Serum AA Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human Serum AA Antibody to each well and incubate for 1 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 15 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.

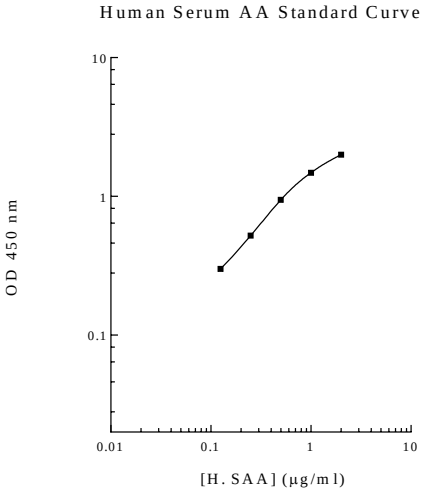
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	µg/ml	OD	Average OD
P1	2.000	2.016 1.984	2.000
P2	1.000	1.435 1.524	1.480
P3	0.500	0.949 0.939	0.944
P4	0.250	0.532 0.513	0.522
P5	0.125	0.304 0.296	0.300
P6	0.000	0.092 0.097	0.095
Sample: Pool Normal, Sodium Citrate Plasma (4x)		1.511 1.509	1.510

Standard Curve

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



Reference Value

- Normal plasma SAA level is less than 6 µg/ml.
- Human plasma and serum samples from healthy adults were tested (n=40). On average, serum AA level was 4.0 µg /ml.

Sample	n	Average Value (µg/ml)
Human Pool Normal Plasma	10	3.8
Human Normal Plasma	20	4.3
Human Pool Normal Serum	10	4.0

Performance Characteristics

- The minimum detectable dose of serum AA as calculated by 2SD from the mean of a zero standard was established to be 0.06 µg/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.1%	3.3%	4.0%	7.7%	8.4%	7.2%
Average CV (%)	3.5%			7.8%		

Spiking Recovery

- Recovery was determined by spiking two plasma samples with different serum AA concentrations.

Sample	Unspiked Sample (µg/ml)	Spike (µg/ml)	Expected	Observed	Recovery (%)
1	0.55	0.15	0.70	0.80	114%
		0.30	0.85	0.89	105%
		1.00	1.55	1.49	96%
2	1.20	0.15	1.35	1.40	104%
		0.30	1.50	1.45	97%
		1.00	2.20	2.16	98%
Average Recovery (%)					102%

Linearity

- Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1:2	91%	92%
1:4	100%	98%
1:8	107%	108%

Cross-Reactivity

Species	Cross Reactivity (%)
Canine	<20%
Bovine	None
Monkey	None
Mouse	None
Rat	<10%
Swine	<20%
Rabbit	None
Human	100%

Notes

- The conversion of IU and mg/ml is 1 International Unit (1IU) = 1.04 mg.

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of expired components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are dry after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.

	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

(1) Malle E et al. (2009) Cell Mol Life Sci. 66(1):9-26
(2) Kluve-Beckerm B *et al.* (1986) *Biochem Genet.* 24(11-12):795-803
(3) Sipe JD *et al.* (1985) *Biochemistry* 24(12):2931-2936.
(4) Watson G *et al*. (1992) *Scand J Immunol.* 36(5):703-712

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