HUMAN SERUM AMYLOID A (SAA) SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: SAA-SP-20

FOR RESEARCH USE ONLY

INTRODUCTION

Serum amyloid A (SAA) is a positive acute phase protein of ~12 kDa that is expressed in the liver and circulates in blood, often bound to lipoproteins. Serum levels can increase >100-fold during the acute phase response, making it a useful biomarker of inflammation and disease.

PRINCIPLE OF THE ASSAY

The human SAA SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses two different peptide specific human SAA antibodies. One is conjugated to horseradish peroxidase (HRP), the other is conjugated to acridan, a chemiluminescent substrate. When the HRP and acridan conjugated antibodies bind to SAA they are brought into close proximity. With the addition of hydrogen peroxide, HRP catalyzes oxidation of proximal acridan molecules causing a flash of chemiluminescence. Acridan conjugated antibodies distant from HRP produce no signal. This principle allows the development of a homogeneous assay that allows rapid measurement of SAA concentrations.

Samples are first treated with dissociation buffer in order to dissociate SAA from lipoproteins. Diluted samples and standards are then mixed with the HRP and acridan-conjugated antibodies in the wells of the 96-well SPARCL™ plate[2] provided with the kit. After incubation for 30 minutes on a shaker at 25°C and 150 rpm, the plate is placed into a luminometer. Trigger solution containing hydrogen peroxide is injected into each well and luminescence is immediately measured. The concentration of SAA is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-SAA HRP conjugate **Store ≤ -70°C**
- Anti-SAA acridan conjugate **Store ≤ -70°C**
- SAA stock[3] **Store ≤ -70°C**
- SAA dissociation buffer, 4.5 ml **Store ≤ -70°C**
- Diluent; CSD50-1, 2 x 50 ml
- Trigger solution; TS7-1, 7 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

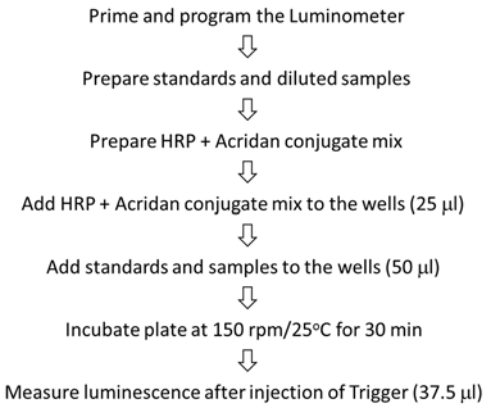
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Curve fitting software

STORAGE

Store the HRP conjugate, acridan conjugate SAA stock and dissociation buffer at -70°C (they may be stored at -20°C for one week). The remainder of the kit should be stored at 2-8°C. The SPARCL™ plate should be kept in a sealed bag with desiccant and antioxidant. The kit will remain stable for at least six months from the date of purchase, provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. Please take the time to completely read all instructions before starting your assay. Contact us if you need clarification.
2. All reagents used in the assay should be allowed to reach room temperature (25°C) before use.
3. It is important that standards and samples be added to the SPARCL™ plate quickly. If testing large numbers of samples, rather than pipetting standards and samples directly into the white SPARCL™ plate using a single channel pipettor, we recommend the following. First, pipette an excess volume of standards and samples into appropriate wells of the clear 96-well plate. Then use an 8- or 12-channel multipipettor to quickly and efficiently transfer 50 µl aliquots to the appropriate wells of the white SPARCL™ plate. The wells of the clear plate have a maximum volume of 300 µl.
4. Follow the sequence of events below when running the assay.



STANDARD PREPARATION

The human SAA stock is comprised of purified human SAA (LDI Cat. No. 9006) in dissociation buffer.

1. Thaw the SAA stock at room temperature.
2. Label 8 polypropylene tubes as 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.195 ng/ml.
3. In the tube labeled 25 ng/ml, prepare the 25 ng/ml standard as described on the SAA stock vial label.
4. Dispense 150 µl of diluent CSD50-1 into the tubes labeled 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.195 ng/ml.
5. Pipette 150 µl of the 25 ng/ml SAA standard into the tube labeled 12.5 ng/ml and mix. This provides the 12.5 ng/ml SAA standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Use the standards within one hour of preparation.

[1] The SPARCL technology was developed by Lumigen Corp.

[2] The plate provided with the kit has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.

[3] The SAA stock is prepared from native human SAA. It behaves identically to monkey SAA. This kit contains no monkey components and is exempt from CITES regulations.

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

SAMPLE PREPARATION

Prior to testing, serum, plasma and fluid samples must be treated with SAA dissociation buffer to dissociate SAA from lipoproteins that interfere with SAA measurement. Use the following procedure for each sample.

Step 1. In a microcentrifuge tube mix 4.0 µl of sample with 36.0 µl of SAA dissociation buffer. Cap the tube and incubate for 20 minutes at room temperature. At this point, the sample has been diluted 10-fold.

Step 2. The dissociated samples must be further diluted at least an additional 100-fold in order to avoid matrix effects attributable to the dissociation buffer. This can be achieved by mixing 2.50 µl of the dissociated SAA prepared in step 1, with 247.5 µl of CSD50-1 diluent. Please note that this represents a 1000-fold dilution of the original sample. If further dilution is needed, use diluent CSD50-1. Do not use other diluents.

Notes. The human SAA SPARCL™ assay uses a homogeneous format and is therefore susceptible to a prozone or "hook effect" at high SAA concentrations. Samples might therefore need to be tested at several dilutions in order to identify and eliminate false low values.

CONJUGATE MIX PREPARATION

Instructions for preparation of the conjugate mix are detailed on the box that contains the HRP and acridan conjugates. If necessary, after thawing, briefly centrifuge to ensure that the contents are at the bottom of the tubes. Prepare the mix shortly before use using the diluent provided with the kit.

LUMINOMETER SETUP

1. The luminometer must be capable of injection and simultaneous measurement of luminescence without any delay.
2. Prime the luminometer injection port with 1 ml of trigger solution.
3. Place the injection needle into the injection port as needed for BMG luminometers.
4. Program the luminometer to inject 37.5 µl of trigger solution per well and to measure from time zero for 1 second (50 x 0.02 second intervals).
5. Define the format of the assay using the luminometer software.
6. Because the white SPARCL™ plate is provided as a 12 x 8-well strips, allowing use of fewer than 96-wells, make sure that the luminometer is programmed to inject trigger solution only into the wells being used.
7. We use a BMG LUMIstar Omega set at a gain of 3600. Optimal gain should be determined by the end user.
8. There are a number of manufacturers of luminometers that are equipped to run a SPARCL™ assay. Please contact Life Diagnostics or Lumigen (www.lumigen.com) to discuss your luminometer.

PROCEDURE

1. Before starting the assay ensure that the luminometer is primed with trigger solution and that the injection needle is positioned in the injection port.
2. Secure the desired number of SPARCL™ 8-well strips in the holder. Immediately seal unused strips in the resealable bag with desiccant and antioxidant. Store unused strips at 2-8°C.
3. Aliquot 25.0 µl of conjugate mix into each well.
4. Dispense 50.0 µl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
5. Incubate on an orbital micro-plate shaker at 150 rpm 25°C for 30 minutes.
6. After the 30-minute incubation, place the plate in the luminometer and measure luminescence after injection of trigger solution (37.5 µl).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

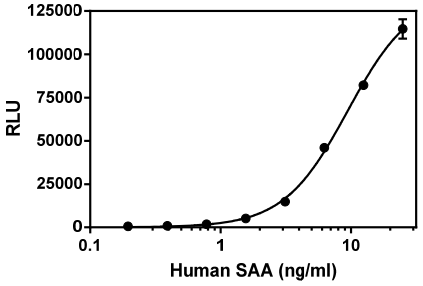
CALCULATION OF RESULTS

1. Before calculating results, review the raw data. If artefacts (RLU spikes) are apparent immediately after injection of trigger solution, eliminate that portion of the luminescence profile from analysis for all wells. We routinely use the sum of RLU values from a 100-980 ms data collection window.
2. Determine the sum of RLU values within the data collection window for the standards and samples.
3. Using graphing software, construct a standard curve by plotting the sum of the RLU values for the standards versus the $\log_{10}$ of SAA concentration and fit to a sigmoidal, 4PL model.
4. Derive the corresponding concentration of SAA in the samples from the standard curve (remember to derive the concentration from the antilog).
5. Multiply the derived concentration by the dilution factor to determine the concentration of SAA in the original sample.
6. If the sum of the RLU values of diluted samples fall outside the standard curve, samples should be appropriately diluted and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. A standard curve should be run with each experiment.

SAA (ng/ml)	RLU
25	79763
12.5	47624
6.25	22605
3.13	7593
1.56	3116
0.48	1436
0.39	760
0.195	448



ASSAY PERFORMANCE

The table below shows results obtained when three human ascites fluid samples were tested in singlets at dilutions ranging from 1000 – 128,000-fold. Coefficients of variation (CV) ranged from 6 – 9%.

Sample	Diln (k)	RLU	Log ng	ng	ug/ml	Av	SD	CV
3	1	128057	1.704169	50.60		60.06	5.73	9.5
	2	110551	1.350347	22.41				
	4	90409	1.151622	14.18	56.71			
	8	47833	0.829223	6.75	53.99			
	16	21866	0.572136	3.73	59.74			
	32	7476	0.277944	1.90	60.69			
	64	2888	0.033672	1.08	69.16			
	128	1226	-0.18371	0.66				
25	1	143570		1.00		93.61	7.71	8.2
	2	133493	2.134861	136.41				
	4	111269	1.359366	22.88	91.50			
	8	71551	1.006785	10.16	81.26			
	16	42230	0.783178	6.07	97.12			
	32	17015	0.499979	3.16	101.19			
	64	5133	0.180504	1.52	96.98			
	128	2406	-0.01266	0.97				
35	1	131793	1.914813	82.19		70.04	7.40	10.6
	2	115398	1.416031	26.06				
	4	97580	1.21386	16.36	65.45			
	8	54538	0.881274	7.61	60.86			
	16	29307	0.661056	4.58	73.31			
	32	9548	0.342377	2.20	70.39			
	64	3718	0.097946	1.25	80.19			
	128	1598	-0.1164	0.76				

REFERENCES

1. Akhavan-Tafti H. et al. A homogeneous chemiluminescent immunoassay method. J Am Chem Soc. 20;135(11):4191-4 (2013)

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For technical support please contact us at
techsupport@lifediagnostics.com