CHICKEN SERUM AMYLOID A (SAA) SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: SAA-SP-5

INTRODUCTION

Serum amyloid A (SAA) is a positive acute phase protein of approximately 12 kDa that is expressed in the liver and circulates in blood. Levels can increase >50-fold in chickens, making it a useful biomarker of inflammation and disease.

PRINCIPLE OF THE ASSAY

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

Diluted samples and standards are mixed with HRP and acridan-conjugates in wells of the SPARCL™ plate[2] provided with the kit. After incubation for 30 minutes 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
- 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 and acridan conjugates at -70°C (they may be stored at -20°C for one week). The remainder of the kit should be stored at 4°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.

Prime and program the Luminometer
⇩
Prepare standards and diluted samples
⇩
Prepare HRP + Acridan conjugate mix
⇩
Add HRP + Acridan conjugate mix to the wells (25 µl)
⇩
Add standards and samples to the wells (50 µl)
⇩
Incubate plate at 150 rpm/25°C for 30 min
⇩
Measure luminescence after injection of Trigger (37.5 µl)

STANDARD PREPARATION

The chicken SAA stock is comprised of pure chicken SAA lyophilized in a stabilizing carrier protein matrix. Reconstitute it as described on the vial label.

1. Label 7 polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.156 ng/ml.
2. In the tube labeled 10 ng/ml, pipette the volume of diluent detailed on the SAA stock vial label. Then add the indicated volume of SAA stock and mix gently. This provides the 10 ng/ml standard. Dispense 150 µl of diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.313, and 0.156 ng/ml.
3. Pipette 150 µl of the 10 ng/ml SAA standard into the tube labeled 5 ng/ml and mix. This provides the 5 ng/ml SAA standard.
4. Similarly prepare the remaining standards by two-fold serial dilution.

Freeze unused stock if future use is intended.

[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.

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

In studies at Life Diagnostics, we found SAA levels ranging from 10 to 1000 ng/ml. Optimal dilutions must be determined empirically but we suggest trying an initial dilution of 200-fold. Only use the CSD50-1 diluent provided with the kit for sample dilution.

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. There must be no 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 several 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 4°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.
5. Incubate on an orbital micro-plate shaker at 150 rpm and 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.

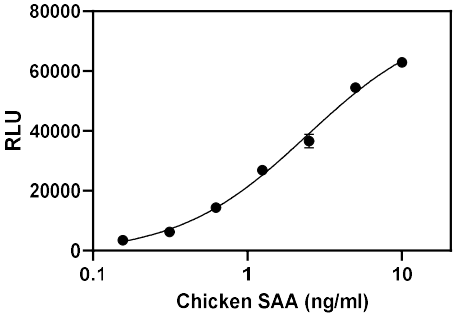
CALCULATION OF RESULTS

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.
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 further diluted and re-tested.

TYPICAL STANDARD CURVE

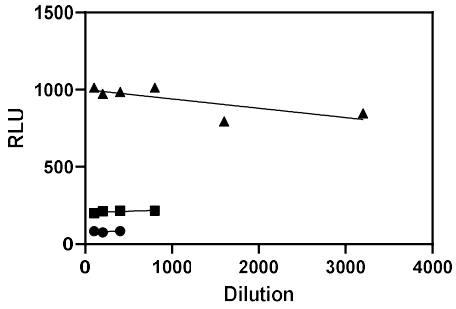
A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. A standard curve should be run with each experiment.

SAA (ng/ml)	RLU
10	62920
5	54494
2.5	36664
1.25	26909
0.625	14399
0.313	6211
0.156	3470



ASSAY PERFORMANCE

Parallelism: To assess performance of the assay, three samples containing SAA at concentrations of 81, 211 and 937 µg/ml were serially diluted from 100- to 3200-fold to produce values within the dynamic range of the assay.



REFERENCES

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

Rev 081821
techsupport@lifediagnostics.com