RAT IgA SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: IGA-SP-2

PRINCIPLE OF THE ASSAY

The rat IgA SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses an affinity purified rat IgA specific antibody. Two conjugates were prepared; one to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugated antibodies bind to IgA 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 determination of IgA concentrations.

The HRP and acridan conjugated antibodies provided with the kit are mixed with standards and diluted samples in wells of the 96-well white SPARCL™ plate provided with the kit[2]. 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 IgA is proportional to luminescence and is derived from a standard curve.

Because the rat IgA SPARCL assay uses a homogenous format, a prozone or hook effect occurs at high IgA concentrations. For this reason, serum or plasma samples should initially be tested at dilutions of 1000 fold. When measuring IgA in other biological fluids or extracts, samples should be tested at a series of dilutions in order to ensure that false low values are not obtained due to either prozone or matrix effects.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-IgA HRP stock **Store ≤ -70°C**
- Anti-IgA acridan stock **Store ≤ -70°C**
- IgA stock **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:

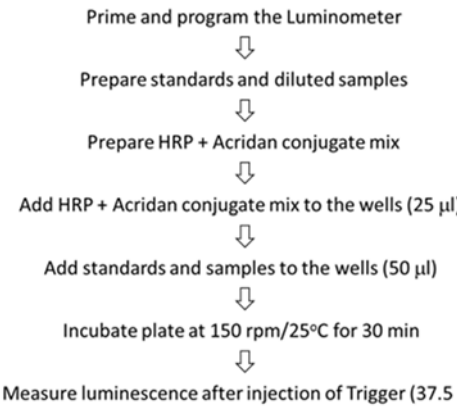
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Curve fitting software

STORAGE

Store the HRP conjugate, acridan conjugate and IgA stock at or below -70°C. 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 hold a maximum volume of 300 µl.
4. Follow the sequence of events below when running the assay.



STANDARD PREPARATION

The IgA stock is comprised of rat serum with a known IgA concentration diluted in a carrier protein matrix[3].

1. Thaw the IgA stock shortly before use.
2. Label 8 polypropylene tubes as 10000, 5000, 2500, 1250, 625, 312.5, 156.25, 78.13 ng/ml.
3. Into the tube labeled 10000 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 10000 ng/ml standard.
4. Dispense 150 µl of diluent into the tubes labeled, 5000, 2500, 1250, 625, 312.5, 156.25, 78.13 ng/ml.
5. Pipette 150 µl of the 10000 ng/ml IgA standard into the tube labeled 5000 ng/ml and mix. This provides the 5000 ng/ml IgA standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Please Note: If future use of the IgA stock is intended, it should be stored frozen at or below -70°C.

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

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

[3]\The IgA used to calibrate the stock was purified at LDI from Sprague Dawley rat serum using salt fractionation and SSL7-agarose chromatography. Its concentration was determined assuming Abs1% at 280 nm = 14.

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SAMPLE PREPARATION

As measured with the rat IgA SPARCL™ assay, we found IgA levels of 2.65±0.78 mg/ml (mean±SD, n=9) in Sprague Dawley rat serum. In order to obtain values within range of the standard curve we suggest that samples initially be tested at a dilution of 1000 fold. This can be accomplished as described below.

1. For each sample to be tested, dispense 495 µl and 225 µl of diluent into separate tubes.
2. Aliquot 5.0 µl of serum or plasma into the first tube and mix to give a 100-fold dilution.
3. Mix 25.0 µl of the 100-fold diluted sample with 225 µl of diluent in the second tube to give a 1000-fold dilution.

In order to avoid matrix effects and false low values, do not test serum or plasma at dilutions lower than 250 fold

CONJUGATE MIX PREPARATION

Instructions for preparation of the conjugate mix are detailed on the box that contains the HRP and acridan conjugates. 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 at least 1 ml of trigger solution.
3. Place the injection needle into the injection port, (necessary 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 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.

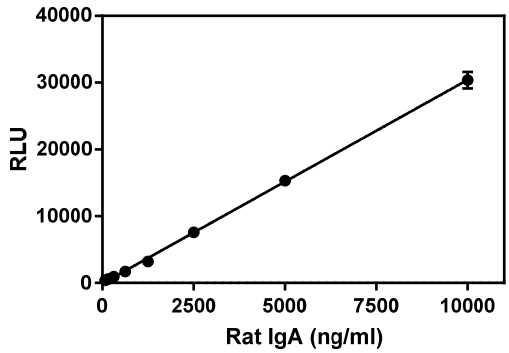
CALCULATION OF RESULTS

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 curve fitting software, construct a standard curve by plotting the sum of RLU values for the standards versus the IgA concentration in ng/ml.
4. Fit the data using a single site, total and nonspecific binding model.
5. Derive the concentration of IgA in the samples from the standard curve.
6. Multiply the derived concentration by the dilution factor to determine the concentration of IgA in the serum or plasma sample.
7. If the sum of RLU values for the samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only. A standard curve must be run with each experiment.

IgA (ng/ml)	RLU
10000	30399
5000	15330
2500	7588
1250	3218
625	1713
312.5	943
156.25	568
78.13	375



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 assistance please email us at techsupport@lifediagnostics.com