RAT ALPHA-1-ACID GLYCOPROTEIN SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: AGP-SP-2

BACKGROUND

Alpha-1-acid glycoprotein (AGP) is a heavily glycosylated protein that is expressed in the liver and secreted into blood. It is a positive acute phase reactant; liver expression and serum concentrations increase as a result of injury, infection or disease. In studies at Life Diagnostics, Inc. we found that serum AGP levels increased approximately 25-fold twenty-four hours after rats were challenged with lipopolysaccharide. AGP is an excellent acute phase biomarker in rats.

PRINCIPLE OF THE ASSAY

The rat AGP SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses two affinity purified AGP specific antibodies. One is conjugated to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugated AGP antibodies bind to AGP 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 AGP 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 AGP is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rat AGP HRP stock. **Store ≤ -70°C**
- Anti-rat AGP acridan stock. **Store ≤ -70°C**
- AGP stock (1 vial). **Store ≤ -70°C**
- Diluent (CSD50-1, 2 bottles)
- Trigger solution (TS7-1)
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

- Precision pipettes and tips
- Polypropylene tubes
- Vortex mixer
- Micro-Plate incubator/shaker
- Luminometer capable of simultaneous injection & measurement
- PC graphing software

STORAGE

Store the HRP conjugate, acridan conjugate and AGP 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 of the 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 a large number 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.

STANDARD PREPARATION

The AGP stock is comprised of pure rat AGP[3] in a carrier protein matrix.

1. Thaw the AGP stock shortly before use.
2. Label 6 polypropylene tubes as 100, 50, 25, 12.5, 6.25 and 3.13 ng/ml.
3. Into the tube labeled 100 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 100 ng/ml standard.
4. Dispense 150 µl of diluent into the tubes labeled 50, 25, 12.5, 6.25 and 3.13 ng/ml.
5. Pipette 150 µl of the 100 ng/ml AGP standard into the tube labeled 50 ng/ml and mix. This provides the 50 ng/ml AGP standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

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

SAMPLE PREPARATION

Using the rat AGP SPARCL™ assay, we found AGP levels of 69±25 µg/ml (mean±SD, n=4) in serum from normal Sprague Dawley rats. Levels increased to 1765±394 µg/ml (mean±SD, n=4) 24 hours after i.p. injection of lipopolysaccharide.

Because the rat AGP SPARCL assay uses a homogenous format, a prozone or hook effect can occur at high AGP concentrations. We found that testing at dilutions of 5,000-, 20,000- and 100,000-fold allowed measurement of AGP in most samples and identification of false low values due to a prozone effect. In order to eliminate matrix effects, serum should not be tested at dilutions below 2,000-fold.

Dilutions of 5,000-, 20,000- and 100,000-fold can be achieved as follows:

[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 rat AGP used in the standard was purified at Life Diagnostics, Inc.

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1. For each sample to be tested, dispense 247.5 µl, 294 µl, 225 µl, and 200 µl of diluent into four tubes.
2. Aliquot 2.50 µl of sample into the tube containing 247.5 µl of diluent and mix to give a 100-fold dilution.
3. Aliquot 6.0 µl of the 100-fold diluted sample into the tube containing 294 µl of diluent and mix to give a 5,000-fold dilution.
4. Aliquot 75.0 µl of the 5,000-fold diluted sample into the tube containing 225 µl of diluent and mix to give a 20,000-fold dilution.
5. Aliquot 50.0 µl of the 20,000-fold diluted sample into the tube containing 200 µl of diluent and mix to give a 100,000-fold dilution.

When measuring AGP in other biological fluids or extracts, samples should be tested at a series of dilutions in order to determine the optimum dilution and to ensure that false low values are not obtained due to either prozone or matrix effects.

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 (CSD50-1).

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 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.

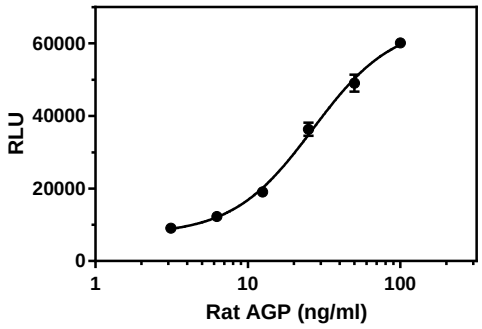
CALCULATION OF RESULTS

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.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus the $\log_{10}$ of the AGP concentration in ng/ml.
3. Fit the data using a variable slope, four-parameter logistic curve.
4. Derive the corresponding concentration of AGP in the samples from the standard curve (derive the concentration from the antilog).
5. Multiply the derived concentration by the dilution factor to determine the actual concentration of AGP in the serum or plasma sample.
6. If the RLU values of diluted samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with RLU plotted on the Y-axis versus $\log_{10}$ AGP concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. A standard curve must be run with each experiment.

AGP (ng/ml)	RLU
100	60102
50	49031
25	36305
12.5	19023
6.25	12299
3.13	9060



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