HUMAN IgG SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: IGG-SP-20

FOR RESEARCH USE ONLY

INTRODUCTION

Pharmacokinetic characterization of human monoclonal IgG antibodies requires measurement of human IgG in serum of species used in preclinical research. This kit allows measurement of human IgG1-4 subclasses[1] in serum of mice, rats, dogs, cynomolgus monkeys and rhesus monkeys.

PRINCIPLE OF THE ASSAY

The human IgG SPARCL™[2] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses a single 30-minute incubation and requires no wash steps. Two conjugates of a human IgG specific antibody are used; one to HRP, the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugated antibodies bind to IgG 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 not bound to IgG produce no signal. This principle allows the development of a rapid homogeneous assay.

The HRP and acridan conjugated antibodies provided with the kit are mixed with standards and diluted samples in wells of the 96-well SPARCL™ plate provided with the kit[3]. 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 IgG is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

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

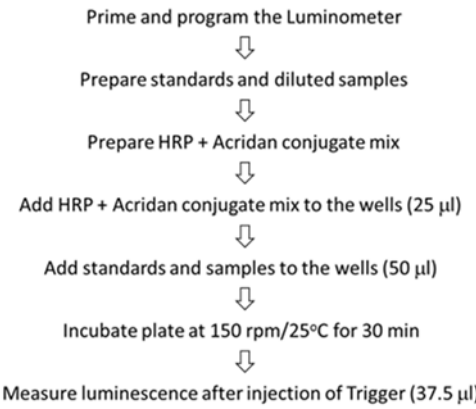
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Curve fitting software

STORAGE

Store the HRP conjugate, acridan conjugate and IgG stock at or below -70°C (they may be stored at -20°C for at least two weeks). 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 appropriately.

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 human IgG stock is comprised of purified human IgG1 diluted in a stabilizing carrier protein matrix.

1. Thaw the IgG stock shortly before use.
2. Label 8 polypropylene tubes as 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/ml.
3. Into the tube labeled 100 ng/ml, pipette the volume of diluent detailed on the IgG stock vial label. Then add the indicated volume of IgG 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, 3.13, 1.56 and 0.78 ng/ml.
5. Pipette 150 µl of the 100 ng/ml IgG standard into the tube labeled 50 ng/ml and mix. This provides the 50 ng/ml IgG standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Use the standards within 30 minutes of preparation. Store unused IgG stock at or below -70°C if future use is intended.

[1] The relative reactivities of IgG(total):IgG1:IgG2:IgG3:IgG4 were determined to be 1.05:1.0:0.25:0.78:0.30.

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

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

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SAMPLE PREPARATION

Because the human IgG SPARCL™ assay uses a homogenous format, a prozone or hook effect can occur at high IgG concentrations. We therefore recommend that samples be tested a several dilutions in order to eliminate false low values. Because the concentrations of human IgG in PK studies depend on the study format it is not possible for us to recommend an optimal dilution factor, they must be determined by the end user. However, all serum samples must be diluted at least 100-fold in order to avoid matrix effects.

Only use the diluent provided with the kit (CSD50-1). Use diluted samples within 30 minutes.

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. Unused conjugate stocks should be returned to the freezer.

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 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.

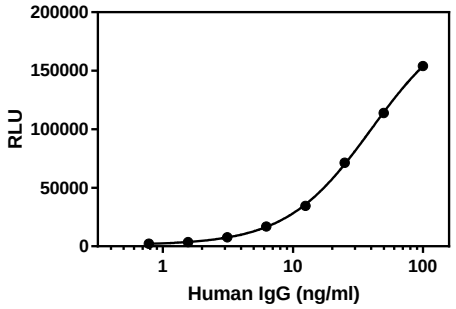
CALCULATION OF RESULTS

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 the IgG concentration and fit to a sigmoidal, 4PL model.
4. Derive the corresponding concentration of IgG 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 IgG 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 with the sum of RLU plotted on the Y-axis versus $\log_{10}$ IgG concentrations on the X-axis is shown below. This curve is for illustration only and should not be used to calculate unknowns. A standard curve must be run with each experiment.

IgG (ng/ml)	RLU
100	154049
50	113889
25	71386
12.5	34645
6.25	16971
3.13	7814
1.56	3548
0.78	2179



ASSAY PERFORMANCE

Spike/recovery data are listed in the table on the next page. Serum from balb/c mouse, sprague dawley rat, mongrel dog, cynomolgus monkey and rhesus monkey was spiked with 500 or 5000 ng/ml of human IgG1. Samples were tested at dilutions of 100, 200, 400… to 3200-fold. Recovery values were 70% or greater. No significant human IgG reactivity was found in unspiked serum from all the above species.

Species	Spike (ng/ml)	Measured (ng/ml)
Mouse	500	423 ± 18
	5000	5064 ± 248
Rat	500	418 ± 53
	5000	4832 ± 261
Dog	500	376 ± 25
	5000	4394 ± 384
Cyno	500	359 ± 37
	5000	4077 ± 663
Rhesus	500	373 ± 41
	5000	4247 ±499

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