LUMIGEN
A Beckman Coulter Company

A Generic Pharmacokinetic Assay using SPARCL

Mark J. Cameron, *Senior Applications Scientist*
Lumigen Inc., a Beckman Coulter Company, Southfield, MI 48033

Introduction:

SPARCL is an acronym for Spatial Proximity Analyte Reagent Capture Luminescence. SPARCL technology is a proximity dependent, non-separation, chemiluminescent detection method. In a SPARCL assay, a chemiluminescent substrate (acridan) is brought into the proximity of an oxidative enzyme (horseradish peroxidase, HRP) through the specific antigen/antibody interaction (Figure 1). A flash of light proportional to the quantity of analyte present in the sample is generated upon addition of a trigger solution containing H_2O_2 and para-hydroxycinnamic acid (pHCA). There is no need to remove excess reactants as SPARCL is a proximity assay.

SPARCL is an immunoassay development tool. It enables the assay developer to rapidly and efficiently develop and validate immunoassays in a variety of formats without the need for specialized equipment beyond the plate based luminometer with injectors. SPARCL assays are very sensitive (based on luminescence), have a wide dynamic ranges, have low minimum required dilution requirements (based on homogeneous format), do not require wash steps and conserve on reagents, and require minimal sample volumes (10-25 uL). Key to a SPARCL assay is the interaction of acridan and HRP in the presence of hydrogen peroxide and ascorbic acid.

Pharmacokinetic (PK) assays measure drug that has been administered to an animal or a human. As part of the drug safety program for each drug and drug candidate, PK assays are and essential and basic part of the evaluation. PK assay provide drug concentration data over time. The SPARCL assay featured here is for measuring a human IgG drug, a “biotherapeutic”. It data shows the utility for using SPARCL for large molecule PK assay development and sample analysis. The assay may be GLP validated in your facility. The generic PK assay shown here is not available as a “kit”. This assay is a sandwich ELISA, the target (or analyte) is human IgG.

The regents featured in this note are commercially available. The SPARCL kit is available from Lumigen, the anti-human IgG antibody is available from SoutherBiotech.

LUMIGEN
A Beckman Coulter Company

Materials and Methods:

Assay Buffer:	PBS with 0.1% BSA (no azide)
Matrix:	Rat serum
"Capture Antibody"	Acridan-labeled clone JDC-10, 125 ug/mL,
"Detection Antibody"	HRP-labeled clone JDC-10 500 ug/mL
"Drug"	human IgG, 10 mg/mL
Trigger Solution	Lumigen's SPARCL kit, hydrogen peroxide
Background Reducing Agent	Antioxidant from Lumigen's SPARCL kit
96 Well Plate	Solid (or break out strips) white, suitable for luminescence,

Assay Preparation Example --- Dilutions:

HRP-labeled JDC-10. Dilute 1:800 in assay buffer. As an example, 3 uL of the HRP conjugate can be added to 2397 uL of assay buffer. Prepare and store at room temperature for a maximum of 4 hours.

Acridan-Labeled JDC-10. Dilute 1:78 in Assay buffer. As an example 38.46 uL can be added to 2961.54 uL of assay buffer. Prepare and store at room temperature for a maximum of 4 hours.

"Drug" (human IgG). Stock is 10 mg/mL. Dilute to 4,000 ng/mL (1:2,500) and then into standard curve. First dilute 1:25 in assay buffer (4 uL plus 96 uL assay buffer for example) and then dilute 1:100 in matrix. Make your dilution series and QC samples in neat matrix.

Assay Preparation --- Mix the 2 Antibody Conjugates Together

Combine equal parts of the diluted HRP conjugated JDC-10 and diluted Acridan Conjugated JDC-10. For example, combine 2 mL and 2 mL of each diluted antibody. Mix and store temperature and use within 4 hours.

Assay Set UP --- Add Reagents to Plate

1. Add 50 uL of antibody mixture (diluted HRP conjugated antibody and diluted acridan conjugated antibody that have been combined for convenience) to desired wells.

LUMIGEN
22900 W 8 Mile Road • Southfield MI 48033 • USA
Telephone: **248 351-5600** • Toll Free: **877 LUMIGEN**
Facsimile: **248 351-0518**

2. Add 25 uL of standards/calibrators/samples to desired wells.
    a. 50 uL of antibody mixture plus 25 uL of matrix = 33.3% matrix in the wells.
3. Cover with plate sealer
4. Shake at medium speed for 30 min, cover with foil.
5. Prime injectors with water (suggest 4 mL)
6. **Important!** Prime injectors with trigger solution (suggest 4 mL)
7. **Important!** Add 4 uL per well of background reducing agent to each well after 30 min incubation has finished.
8. Position plate on plate reader.
9. Inject 75 uL of trigger per well and read immediately upon injection.

LUMIGEN
22900 W 8 Mile Road • Southfield MI 48033 • USA
Telephone: **248 351-5600** • Toll Free: **877 LUMIGEN**
Facsimile: **248 351-0518**

LUMIGEN
A Beckman Coulter Company

Suggested Luminometer Set Up for SPARCL Assays

SPARCL Assays are fast kinetic assays. The flash of light is generated immediately upon injection of the trigger solution and peaks at 0.15 seconds and rapidly decays over 1 second. The luminometer must be able to read the flash of light at the time of injection and without delay. The following is an example of how Softmax can be set up for SPARCL assays. Consult with Lumigen or the maker of your plate reader for other software packages. The information provided is when using SoftMax Pro 5.4

Mode:	Fast Kinetic, Luminescence
Integration Point/Count:	Integrate 0.02 seconds, repeat 50 times (total read time is 1 second)
Sensitivity:	Photon counting, correction is set to none
Automix:	Not used
Assay Plate Type:	96-well Standard
Wells to Read:	You need to enter in the well information based on your needs
Injection and Delay:	P-injection is set to "off"
	M-Injection volume of 75 uL, baseline read delay is set to "zero",
	Injection speed set to 230 uL/second, Shake after injection is set to "zero", number of baseline reads is set to "zero"
Injection Wells:	You need to enter the well information based on your needs
Dark Adaptor:	Off
AutoRead	Off

LUMIGEN
22900 W 8 Mile Road • Southfield MI 48033 • USA
Telephone: **248 351-5600** • Toll Free: **877 LUMIGEN**
Facsimile: **248 351-0518**

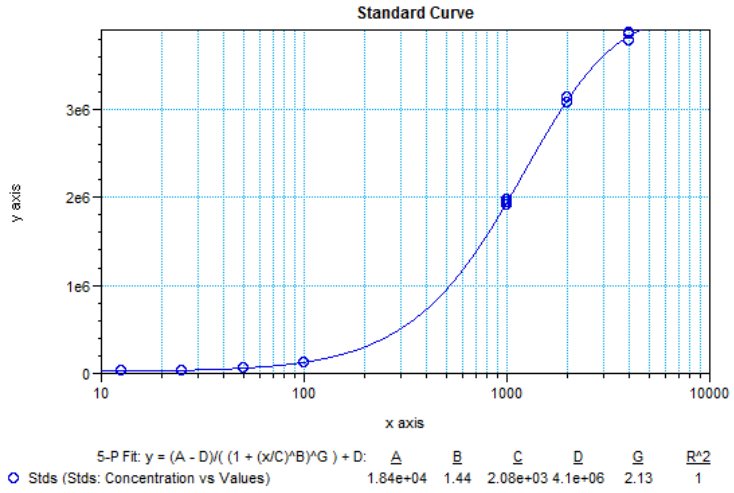
LUMIGEN
A Beckman Coulter Company

RESULTS:

A typical standard curve is represented in figure 2. The assay has a wide dynamic range (12.5-4,000 ng/mL). Rat serum samples are applied neat to the assay. As a result, the minimum required dilution (MRD) is "neat". Table 1 summarizes the inter assay performance. The greatest total error for any QC level is 11.3%. Total error represents the sum (absolute value) of the precision and accuracy.

CONCLUSION:

SPARCL enables rapid and sensitive PK assay development that is cost effective with remarkable perfomance.



Stds (ng/ml)

Sample	Concentration	Wells	Values	MeanValue	Std.Dev.	CV%	Back Calc
Gr01	4000	A2	3853951	3829598	51527.3	1.3	4000.1
		A3	3864435				
		A4	3770408				
Gr02	2000	B2	3077585	3113166	30915.9	1.0	2000.0
		B3	3128440				
		B4	3133472				
Gr03	1000	C2	1945791	1942815	27578.6	1.4	1000.0
		C3	1968785				
		C4	1913869				
Gr04	100	D2	126822	125493	1611.2	1.3	99.4
		D3	123701				
		D4	125956				
Gr05	50	E2	59439	59700	691.7	1.2	50.9
		E3	60484				
		E4	59176				
Gr06	25	F2	34771	34227	515.0	1.5	26.0
		F3	34163				
		F4	33747				
Gr07	13	G2	24220	25089	753.6	3.0	14.3
		G3	25478				
		G4	25567				
Gr08	0	H2	15536	16243	749.7	4.6	Range?
		H3	16164				
		H4	17029				

Figure 2. A typical standard curve and data.

LUMIGEN
22900 W 8 Mile Road • Southfield MI 48033 • USA
Telephone: **248 351-5600** • Toll Free: **877 LUMIGEN**
Facsimile: **248 351-0518**

Characteristic	Statistic	QC 1000	QC 100	QC 50	QC 25
	Mean				
# Results	N	17	17	17	17
Accuracy	Mean Bias (%RE)	3.9	-1.1	0.4	2.2
Precision	%CV	7.0	4.0	7.9	9.1
Total Error	%RE + %CV	10.9	5.1	8.3	11.3
(Accuracy + Precision)					

Table 1. Inter Assay Performance

Additional Information:

Please contact technical@lumigen for assistance.

LUMIGEN
22900 W 8 Mile Road • Southfield MI 48033 • USA
Telephone: **248 351-5600** • Toll Free: **877 LUMIGEN**
Facsimile: **248 351-0518**