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SPARCL

A Sensitive and Cost Effective Homogeneous Immunoassay Technology

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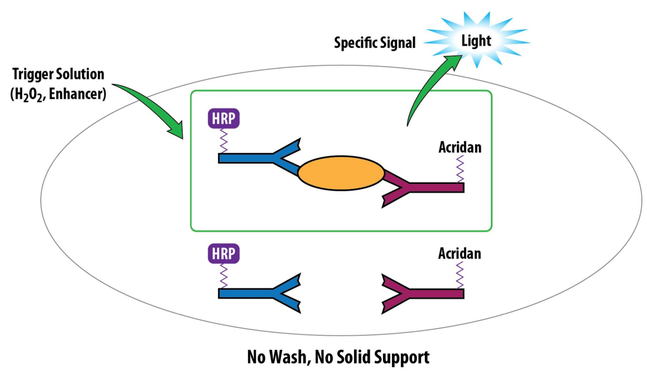
Abstract

SPARCL™ (Spatial Proximity Analyte Reagent Capture Luminescence) technology enables no-wash immunoassays featuring the use of peroxidase induced chemiluminescence for quantification of an analyte based on close proximity. This assay technology, applicable to both sandwich and competitive immunoassays, uses a chemiluminescent compound (acridan) labeled binding partner in conjunction with an HRP-conjugated antibody or antigen molecule. The specific antigen-antibody interaction brings the acridan and HRP into close proximity. A flash luminescence is then generated upon addition of a trigger solution.

Examples of several commonly used assays are described to show the utility, assay performance, and broad applicability of SPARCL™ technology. The unique features of this new technology allow for rapid assay development and optimization using readily available reagents. It provides high sensitivity as well as great potential in reducing cost

SPARCL™ Technology Principle

SPARCL™ (Spatial Proximity Analyte Reagent Capture Luminescence) technology is a proximity dependent, non-separation chemiluminescent detection method. In a SPARCL™ assay a chemiluminescent compound (acridan) is brought into close proximity to an oxidative enzyme (horseradish peroxidase, HRP) through specific antigen/antibody interaction. A flash of light proportional to the quantity of analyte present in the sample is generated upon addition of a trigger solution containing hydrogen peroxide. A background reducing agent (BGR) is often used to enhance S/N ratios. There is no need to remove excess reactants.



No Wash, No Solid Support

Lumigen SPARCL™ Detection Kit

A: Lumigen SPARCL Labeling Reagent
- Activated NHS ester of an acridan compound

B: Lumigen SPARCL BGR
- Background reducing agent used to enhance S/N ratios

C: Lumigen SPARCL Trigger Solution
- Hydrogen peroxide containing solution for signal generation

** Also included in the kit is a Borate Buffer Pack for the labeling reaction*

Typical SPARCL™ Assay Workflow

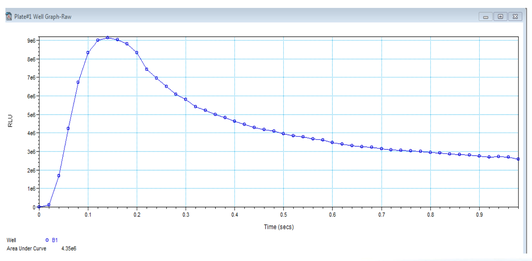
Step 1: Label one of the antibody with the activated SPARCL label. No purification required.

Step 2: Add antibody solutions and sample; Incubate for 30 minutes

Step 3: Add SPARCL BGR

Step 4: Read the luminescence signal by injecting SPARCL Trigger Solution on a luminescence plate reader

Typical SPARCL™ Flash Luminescence Signal



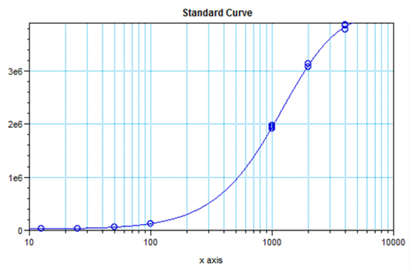
A Generic Pharmacokinetic (PK) Assay for Antibody Drugs

Pharmacokinetic (PK) assays provide drug concentration data over time after administration and are an essential part of biological drug development programs. The SPARCL assay featured here is for measuring a human IgG drug, a "biotherapeutic". For comparison purposes, the assay uses the same antibodies described in a published paper[1].

Materials:
Assay Buffer— PBS with 0.1% BSA, **Sample Matrix**— Rat serum; **"Capture Antibody"**— Acridan-labeled mouse anti human IgG clone JDC-10 (working solution at 1.60 µg/mL); **"Detection Antibody"**— HRP-labeled mouse anti human IgG clone JDC-10 (working solution at 1.60 µg/mL); **"Drug"**— human IgG, 10 mg/mL; **SPARCL BGR and Trigger Solution**.

Assay Methods:
1) Add 50 µL of antibody mixture (1:1) (working solutions of HRP conjugated antibody and acridan conjugated antibody that have been combined for convenience) to desired wells; 2) Add 25 µL of standards/samples; 3) Cover with plate sealer and shake at medium speed for 30 min at room temperature (covered with foil); 4) Add 4 µL per well of SPARCL BGR to each well; 5) On a SpectraMax-L plate reader inject 75 µL of SPARCL Trigger solution per well and read immediately for one second upon injection.

Results :



LLOQ: 12.5 ng/mL, ULOQ: 4000 ng/mL

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				

Assay Variability-Accuracy and Precision

Characteristic	Statistic	QC 1000	QC 100	QC 50	QC 25
# Results	Mean (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	11	5.1	8.3	11

Comparison with Literature Data[1]

Platform	LBA format	Readout	Dynamic range (ng/mL)	Sensitivity (ng/mL)	Sample MRD	Required sample volume (µL)	Total assay time (h)	Qualification status	Comments
ELISA	Plate	Colorimetric	88.0 – 666	88.0	20	12	5	✔	Assay time does not include O/N coat
MSD	Plate	ECL	15.6 – 4000	15.6	4	25	2	✔	Uses pre-coated SAv plates
Gyrolab	Bead	Fluorescence	10.5 – 6400	10.5	2	4	1.5	✔	Assay time is per 96 data points
AlphaLISA	Bead	Luminescence	181 – 1097	181	N/A	10	2.5	X	Hook effect observed
SPARCL	Plate	Luminescence	12.5 - 4000	12.5	neat	25*	0.5	NA	Homogeneous

*SPARCL Assays are flexible and sample volume can be adjusted

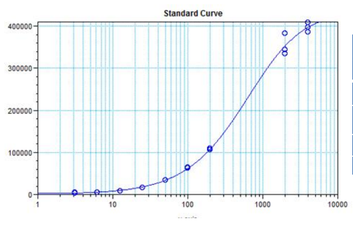
Reference: 1) "Bioanalytical platform comparison using a generic human IgG PK assay format" Beth A. Leary, et al. Journal of Immunological Methods 397 (2013) 28–36

SPARCL Human IL-8 Assay in Cell Culture Media and Plasma

Materials:
Assay Buffer— PBS with 0.05% Tween-20 and 0.1% BSA; **Sample Matrix**— RPMI-1640 (Sigma, catalog number R8758) containing 10% fetal bovine serum (Sigma, catalog number F4135), or assay buffer containing 10% human plasma (Bioreclamation); **"Capture Antibody"**— Acridan-labeled mouse anti-human IL-8 antibody (R&D Systems, catalog number MAB208, working solution at 0.25 µg/mL), **"Detection Antibody"**— HRP-conjugated mouse anti-human IL-8 (Anogen, catalog number MO-C40017T, working solution at 0.15 µg/mL); **"Analyte"**— recombinant human IL-8 (R&D Systems, catalog number 208-IL-010); **SPARCL BGR and Trigger Solution**.

Assay Methods:
1) Add 50 µL of antibody mixture (1:1) and 25 µL of standards/samples to each well; 2) Cover with plate sealer and shake at medium speed for 60 min (covered with foil); 3) Add 5 µL per well of background reducing agent; 4) On a SpectraMax-L plate reader inject 75 µL of SPARCL Trigger solution per well and read immediately upon injection.

Results :



LLOQ: 3.1 pg/mL, ULOQ: 4000 pg/mL

Analytical Sensitivity: 1.1 pg/mL (3 SD)

Inter Assay Performance in 10% Plasma

Characteristic	Statistic	QC 200	QC 100	QC 50	QC 25	QC 12.5	QC 6.25
# Results	Mean (N)	6	6	6	6	6	6
Accuracy	Mean Bias (%RE)	0.8	3.5	0.8	3.6	4.8	2.4
Precision	%CV	6.1	3.4	1.9	11	4.9	7.7
Total Error	%RE + %CV	6.9	6.9	2.7	15	9.7	10

Inter Assay Performance in Cell Culture Medium

Characteristic	Statistic	QC 1000	QC 100	QC 50	QC 25	QC 12.5	QC 6.25
# Results	Mean (N)	6	6	6	6	6	6
Accuracy	Mean Bias (%RE)	2.5	0.3	0.5	0.5	0.2	0.1
Precision	%CV	16	3.2	4.2	2.2	4.2	8.2
Total Error	%RE + %CV	18	3.5	4.7	2.7	4.4	8.3

IL-8 Assay Linearity of Dilution

Theoretical (pg/mL)	Measured (pg/mL)	Dilution Factor	Percent of Expected
5000	4810	10	NA
500	458	2	95
250	217	2	95
125	104	4	96
31.25	25	2	96
15.6	12.6	2	100
7.8	7.1	NA	112

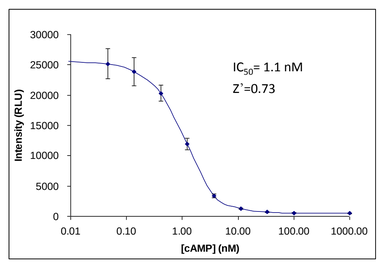
Note: *IL-8 standard (5000 pg/mL) spike into 10% matrix exposed to dilution series*

cAMP Assay: A Competitive SPARCL Immunoassay

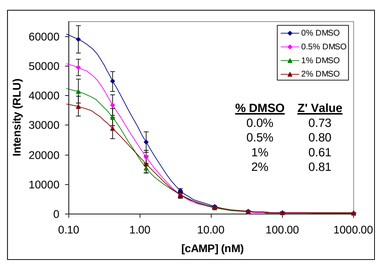
Materials and Methods:
1) **Anti-cAMP Antibody** 6 µL; **cAMP-HRP** 6 µL; **cAMP Calibrator** 4 µL; **SPARCL BGR** 2 µL
2) 45-minute incubation at room temperature; 3) **SPARCL Trigger** 14 µL and read (SpectraMax-L, 1 second) *Note: miniaturized assay tailored for high-through-put in a 384 well plate.*

Results:

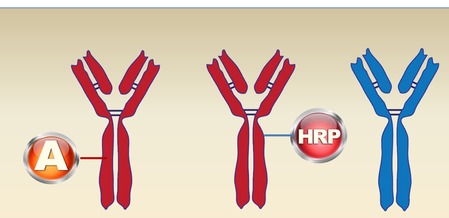
Standard Curve



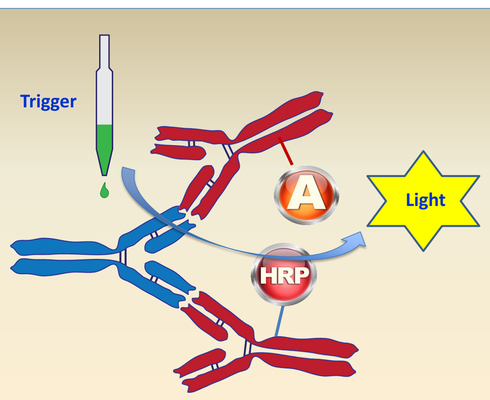
DMSO Tolerance



SPARCL Applications in Immunogenicity



Basic components of SPARCL immunogenicity assay:
An acridan labeled antibody drug (red), a HRP labeled antibody drug (red) and an anti-drug antibody (in blue).



A SPARCL bridging assay for Immunogenicity: formation of a bridge results in proximity of acridan and HRP.

Conclusions

- SPARCL™ is a flexible assay development tool that can be utilized and implemented in different immunoassay formats and numerous application areas:
  - sandwich and competitive
  - quantitative and qualitative
  - PK, biomarker, immunogenicity and HTS
  - ELISA/MSD/Gyros conversions to a cost effective strategy
- SPARCL™ assays exhibit outstanding performance:
  - wide dynamic range
  - high sensitivity
  - low MRD
  - simple, fast and easy
- SPARCL™ enables a cost-effective approach:
  - cost savings in every aspect of assay development, validation and production
  - short run times, conservation of reagents and samples
  - labor, consumables, waste disposal, capital equipment and service contracts