

Using SPARCL technology to Develop Immunoassays for Biomarker Detection and Pharmacokinetic Studies

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- The SPARCL assay provides a platform for simplified development of immunoassays
- The reading at time of injection capability of the FLUOstar Omega allows the assessment of the entire SPARCL signal

Introduction

Biomarkers are used in diverse scientific fields to evaluate normal and pathogenic processes or the response to therapy. Here we look at the biomarker interleukin 8 (IL-8) which is often associated with inflammation and has been shown to be increased by oxidative stress.

Pharmacokinetic (PK) assays are used to measure the concentration of a drug that has been administered to a human or animal. PK assay data are used to evaluate elimination profiles of the drug and are important in drug safety and toxicology studies.

In this application note we will show how the performance of the FLUOstar Omega microplate reader enables the detection of SPARCL (Spatial Proximity Analyte Reagent Capture Luminescence) assays. Examples of biomarker detection and pharmacokinetic assays are presented.

Assay Principle

SPARCL detection technology was used for rapid immunoassay development. This technology is a proximity dependent and homogeneous method where an antibody/antigen interaction leads to proximity of a chemiluminescent substrate (acridan, bound to antibody 1) and an oxidative enzyme (horseradish peroxidase [HRP, bound to antibody 2]). Introduction of a trigger solution containing H₂O₂ generates a flash of light proportional to the amount of analyte present (Figure 1).

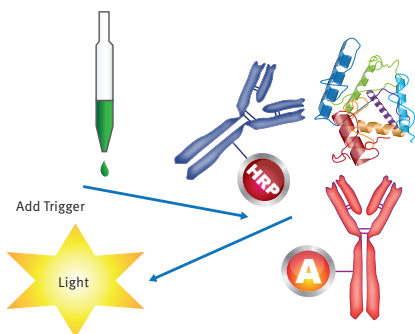
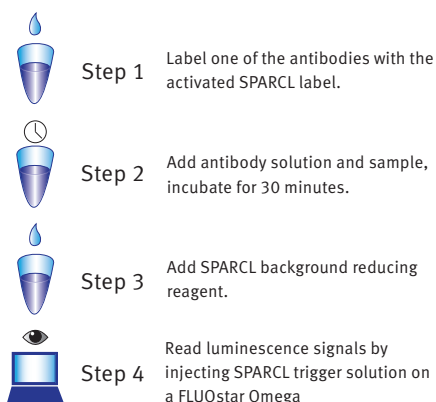


Fig. 1: Representative SPARCL Assay. Specific antibody/antigen interactions bring acridan and HRP into close proximity. The H₂O₂ based trigger solution, which is added subsequently, interacts with acridan (A) and HRP causing a flash of light.

Materials & Methods

- SPARCL kits (Lumigen)
- FLUOstar Omega microplate reader (BMG LABTECH)
- 96-well, white, flat bottom microplates from Greiner
- Other reagents were obtained from commercial sources

A typical Lumigen SPARCL assay workflow is shown below.



For each experiment one antibody was labeled with acridan according to SPARCL kit instructions while a second HRP-labeled antibody was purchased.

Biomarker Assay

A 1:2 dilution series of IL-8 was prepared from a starting concentration of 1000 pg/mL. Dilution of controls and the dilution series were made in cell culture media or PBS containing 0.1% BSA and 10% human plasma.

Pharmacokinetic Assay

A standard curve was generated using a dilution series of human IgG in neat (undiluted) rat serum. The highest point in the standard curve was 1000 ng/mL. Quality Control samples (QC) were made by spiking human IgG drug into neat rat matrix. QC concentration values were interpolated from the standard curve. The interpolated QC sample values were used to determine accuracy, precision and total error.

For both experiments sample plating, antibody addition, incubation and addition of background reducing agent were performed according to instructions.¹ The plates were then read on the FLUOstar Omega plate reader with the following settings which include injection of the trigger solution.

FLUOstar Omega instrument settings

Measurement type:	Luminescent (well mode)
No. of intervals:	50
Interval time:	0.02 s
Measurement interval time:	0.02 s
Emission filter:	lens
Gain:	3600
Injection volume:	75 µl
Pump speed:	300 µl/s

Results & Discussion

The FLUOstar Omega features reading at time of injection which allows for the assessment of signal produced in the SPARCL reaction upon addition of the trigger solution (Figure 2).

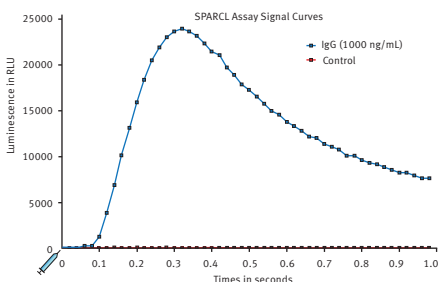


Fig. 2: Typical SPARCL Pattern of Flash Luminescence. Light energy was captured every 0.02 seconds for 1 second. This representative curve contains 1000 ng/mL of human IgG. The control does not contain IgG.

Biomarker Assay

The data produced by the SPARCL IL-8 biomarker experiment was evaluated using results based on calculating the area under the curve. As seen in Figure 3 this method is suitable for assessing the SPARCL reaction providing a reliable fit curve.

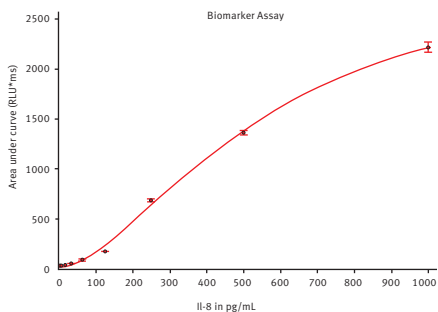


Fig. 3: 4-Parameter Fit Curve of SPARCL IL-8 Data. Data calculated using area under the curve conforms to a 4-parameter fit curve with an R2 value of 0.998.

Pharmacokinetic Assay

Similarly SPARCL PK assays using IgG as a "drug" were performed. Figure 4 shows the representative curve produced from a dilution series of human IgG.

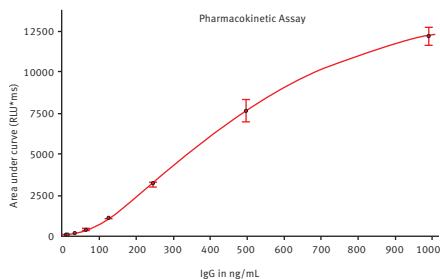


Fig. 4: 4-Parameter Fit Curve of SPARCL PK Data. Data calculated using area under the curve conforms to a 4-parameter fit curve with an R2 value of 0.99996.

Assay precision and reproducibility were assessed for the PK assay using replicates of QC samples tested in 2 different SPARCL assay test runs as shown below (Table 1).

	QC500	QC125	QC31.25
Number of measurements	6	6	6
Accuracy [%RE]*	-0.5	-3.7	-7.6
Precision [%CV]**	8.1	2.9	4.9
Total Error [%RE +%CV]	8.6	6.6	12.5

*%RE = Percent relative error; **CV = coefficient of variation

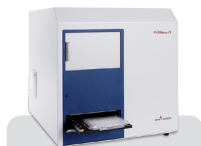
Conclusion

Lumigen's SPARCL assay enables simplified immunoassay development when paired with the reading at time of injection capabilities of BMG LABTECH microplate readers such as the FLUOstar Omega. Both the IL-8 and the PK SPARCL assays show acceptable accuracy, precision and total error. SPARCL may be formatted for many different assay types including high throughput screening applications.

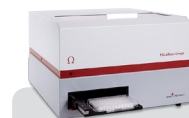
References

1. For more information about SPARCL contact Lumigen at SPARCL@beckman.com or visit www.lumigen.com/detection-technologies/sparcl

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