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Development of an IL-8 Assay in Tissue Culture Media and Plasma Using SPARCL™ Technology

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INTRODUCTION

A homogeneous chemiluminescent human IL-8 assay has been developed based on SPARCL™ technology. The assay uses two antibodies conjugated respectively with horseradish peroxidase (HRP) and an acridan compound. The acridan compound and HRP are brought into close proximity as a result of the specific antigen-antibody interaction. Quantification of IL-8 is achieved by measuring the light signal that is generated upon addition of a trigger solution containing H_2O_2 without the need to remove excess reactants.

MATERIALS AND INSTRUMENTATION

Lumigen SPARCL™ Detection Kit: (Lumigen, Inc. Catalog No. SDK-10K) Included in the kit are SPARCL Labeling Reagent, BuHTM Borate Buffer Pack, SPARCL Background Reducing Agent (BGR) and SPARCL Trigger Solution.
Mouse Monoclonal Anti-human IL-8 Antibody: Purchased from R&D Systems (Catalog Number MAB208).
HRP-Conjugated Mouse Monoclonal Anti-human IL-8 Antibody: Purchased from Anogen, a division of YES Biotech Laboratories (Catalog number MO-C40017T).
Recombinant Human IL-8: Purchased as a lyophilized powder from R&D Systems (Catalog Number 208-IL-010).
Assay Buffer: 1xPBS containing 0.05% Tween-20 and 0.1% BSA.
Mammalian Cell Culture Media: RPMI-1640 (Sigma, Catalog Number R8758) containing 10% fetal bovine serum (Sigma, Catalog Number F4135).
Human Plasma: Purchased from Bioreclamation (Catalog Number HMPLNAHP)
Luminescence Plate Reader: SpectraMax L (Molecular Devices) (Note: FLUOstar Omega from BMG could also be used)

ASSAY PROTOCOL

1) Prepare acridan-labeled mouse monoclonal anti-human IL-8 antibody following standard procedure indicated in Lumigen SPARCL™ Detection Kit.
2) Prepare working solutions of acridan-labeled mouse anti-human IL-8 antibody (1:1000 from the labeling reaction mixture) and HRP-conjugated mouse monoclonal anti-human IL-8 antibody (1: 3333 from the commercial stock) by serial dilution with the assay buffer.
3) Prepare 1:1 mixture solution by combining equal volumes of the two antibody working solutions from Step 2.

4) Prepare IL-8 standards or controls from recombinant human IL-8 by serial dilution in cell culture media or PBS containing 0.1% BSA and 10% human plasma.
5) To each well of a 96-well plate, add 50 µL of antibody mixture solution and 25 µL of IL-8 standard or control.
6) Incubate at room temperature for 60 minutes.
7) Add 5 uL of SPARCL BGR to each well.
8) Read the plate on SpectraMax-L by injecting 75 µL of SPARCL Trigger Solution to each well (total read time: 1 second; 0-sec delay between injection and read; Note: FLUOstar Omega from BMG could also be used)

RESULTS

A SPARCL™ IL-8 assay can be readily developed using commercially available antibodies and Lumigen SPARCL Detection Kit. The assay development process starts with labeling one of the two antibodies with an acridan compound, a straightforward step with no need for purification due to uniqueness of the SPARCL technology. The "Add-Incubate-Add-Read" workflow as indicated in the protocol shown above makes assay development an easy task even for users who are new to SPARCL technology.

The SPARCL™ IL-8 assay developed here as an example to demonstrate the applicability of SPARCL technology, displays excellent performance both in terms of sensitivity and variability. Figure 1 shows a typical standard curve with an LLOQ of 3.1 pg/mL and ULOQ of 4000 pg/mL in cell culture media or 10% plasma. The analytical sensitivity in cell culture media is calculated to be 1.1 pg/mL using 3 standard deviations from the standard blanks (16 replicates). The assay also exhibits good accuracy and precision (Table 1 and 2), and excellent dilution linearity as well (Table 3).

A comparison to several commercially available IL-8 assays is summarized in Table 4. In addition to simplicity the SPARCL™ assay data shows better sensitivity and dynamic range with significantly reduced total assay time.

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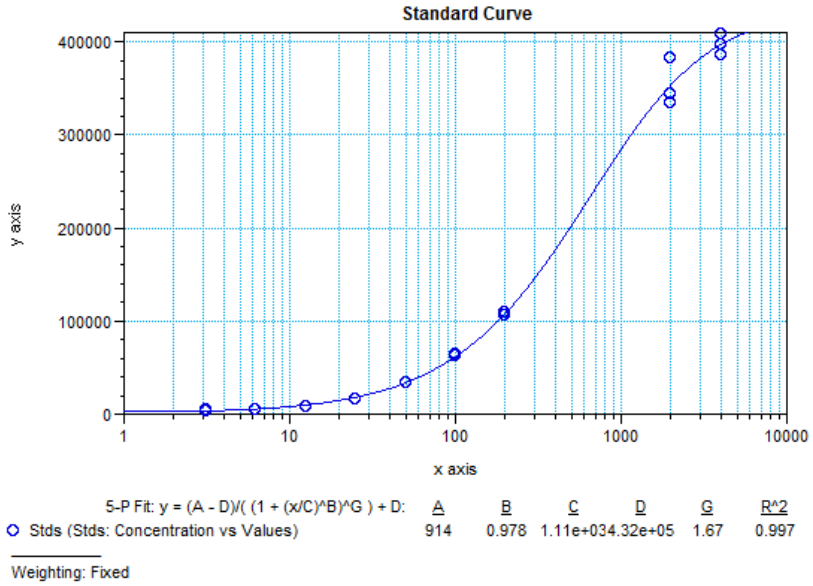

Figure 1. Typical Standard Curve

Table 1. Inter Assay Performance in Cell Culture Medium: RPMI 1640 with 10% FBS

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	15.7	3.2	4.2	2.2	4.2	8.2
Total Error	%RE + %CV	18.2	3.5	4.7	2.7	4.4	8.3

Table 2. Inter Assay Performance in 10% Human Plasma in PBS + 0.1% BSA

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)	0.75	3.5	0.8	3.6	4.8	2.4
Precision	%CV	6.1	3.4	1.9	11.1	4.9	7.7
Total Error	%RE + %CV	6.9	6.9	2.7	14.7	9.7	10

Table 3. Linearity of Dilution

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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

* IL-8 standard (5000 pg/ml) spike into 10% matrix exposed to dilution series.
"Percent of Expected" is based on measured values after dilution.

Table 4. IL-8 Assay Comparison

**Data for commercial assays are obtained from product brochures on the related company's website*

	Format	Assay Duration	Sensitivity (pg/ml)	Concentration Range (pg/ml)
SPARCL	Homogeneous	1 hr	1.1	3.0 - 4000
CisBio	Homogeneous	2 hr	13	20 - 2000
Life Technologies (Novex)	ELISA	2.5 hr	5	15.6 - 1000
R&D Systems (Quantikine)	ELISA	3.5 hr	7.5	31.2 - 2000

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