

Lumigen SPARCL

No-Wash Homogeneous Immunoassay

Lumigen SPARCL is a homogeneous proximity assay technology utilizing flash chemiluminescence detection without solid support or wash steps allowing for assays to be completed in less than 30 minutes. The SPARCL technology enables assays to be miniaturized for high throughput screening while maintaining sensitive results with good dynamic range. The solution phase kinetics of SPARCL mimic the native in vivo environment by

eliminating variability inherent in attachment to solid phase producing faster, more accurate results. SPARCL can be automated and adapted for multiple applications including ELISA, protein-protein and protein-nucleic acid interactions, and high throughput binding assays. Replace existing assays or implement new assays with this breakthrough technology and move into the future of high throughput screening.

Complete assays in less than 30 minutes

Save time with only one incubation step for antigen-antibody binding, no separation or wash steps and instantaneous signal generation from flash chemiluminescence

Cost effective

Use fewer reagents, smaller amounts of samples and fewer instruments with no need for plate/bead coating operations, automated washing stations or other expensive detection equipment.

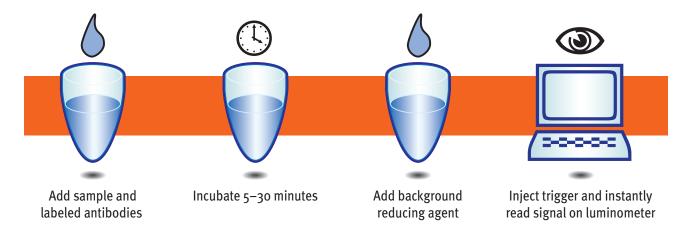
Flexible assay formats

Adaptable for many different kinds of assays in solution or solid phase formats to study a wide variety of targets

Produce less waste

No wash means no disposal of wash solution. SPARCL produces only 1x well volume of waste compared to 13x well volume for conventional ELISA assays.

4 Steps - 30 minutes - No solid support - No wash



SPARCL vs Conventional ELISA Assays

Homogeneous Immunoassay

Microtiter well plate and bead based ELISA assays involve two sequential antibody binding events for the specific binding of the analyte of interest to the capture antibody as well as the specific binding of the same analyte to an enzyme labeled antibody for detection.

Each binding event takes time (30 minutes to 1 hour) and each binding step must be followed by wash steps to remove unbound and non-specifically bound materials. Washes after each antibody binding event are usually repeated 2-3x to ensure that all unbound and loosely bound non-specific materials have been removed.

In SPARCL assays the two binding events between the analyte of interest and the two analyte specific antibodies occur simultaneously.

The unbound materials have no effect on the outcome of the assay eliminating the second binding step and all wash steps.

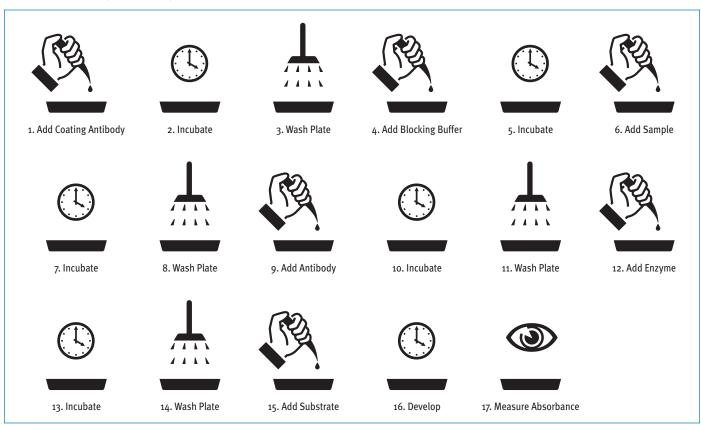
Solid Phase

Tethering of a capture molecule to a solid support is essential for an ELISA assay as this enables the separation of specifically bound molecules from those that are not bound. Tethering is not required in a SPARCL assay.

Signal Generation

Conventional ELISA assays require the substrate and enzyme to develop for up to 30 minutes before measuring absorbance. In SPARCL assays a flash of light proportional to the quantity of analyte present in the sample is instantly generated upon addition of a trigger solution.

Standard ELISA (Sandwich)



SPARCL Assay (Sandwich)

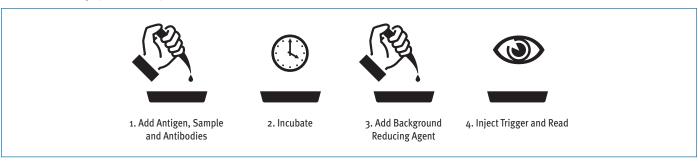


Figure 1. Comparative workflows between conventional ELISA assays and SPARCL assays.

SPARCL Technology

Spatial Proximity Analyte Reagent Capture Luminescence (SPARCL) is a proximity dependent chemiluminescent technology for the detection of specific binding interaction or association between two binding partners. In a SPARCL assay, a binding partner labeled with a chemiluminescent substrate (acridan) and a second binding partner labeled with horseradish peroxidase

(HRP) are brought into close proximity to each other through a specific binding event. Because of this close proximity of acridan to the HRP enzyme a flash of chemiluminescence is generated upon addition of a trigger solution containing hydrogen peroxide and an enhancer.

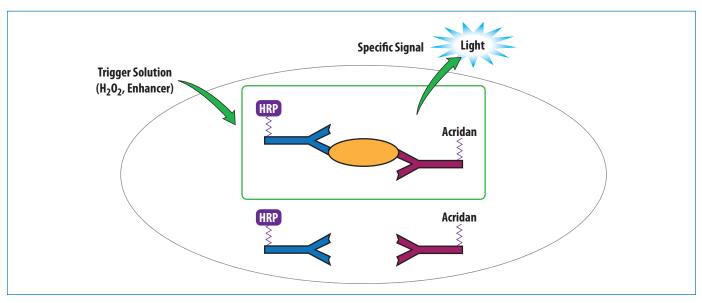


Figure 2. A Simplified Mechanistic Scheme of Spatial Proximity Analyte Reagent Capture Luminescence (SPARCL) Technology

SPARCL Applications

SPARCL can be used for multiple applications for the detection of specific binding interactions or association between two binding partners including:

- ELISA assays
 - Sandwich
 - Direct
 - Indirect
 - Competitive
- Protein-protein, protein-antibody, protein-DNA, DNA-DNA interactions
- High throughput binding assays
- · Cell based assays

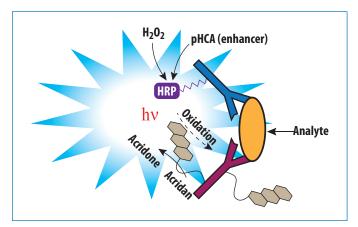


Figure 3. Sandwich Assay - Solution Phase

SPARCL Formats

Solution or solid phase assay

SPARCL can be implemented in formats with or without a solid phase. When using a solid phase, both the acridan compound and a specific capture antibody are coupled to solid phases such as micro particles or microtiter plates, whereas when the solid phase is omitted the capture antibody is directly labeled with the acridan compound. The solution phase format eliminates the need for plate or particle coating, improves kinetics to shorten incubation time and due to the lack of a solid/solution interface offers a more native biological environment.

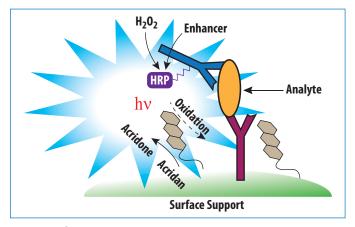


Figure 4. Solid Phase Assay

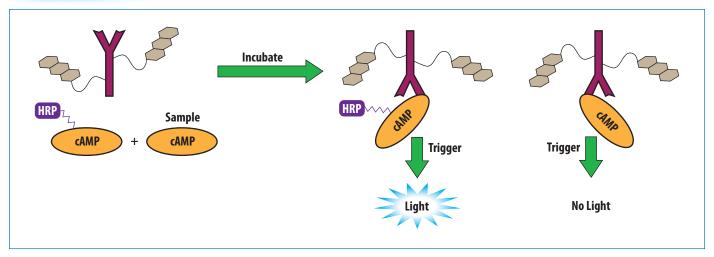


Figure 5. Competitive Assay - Solution Phase



Product Specifications

Enzyme	Horseradish Peroxidase (HRP)
Application	ELISA, Proximity Assays
Sensitivity	Low picogram
Signal Duration	Instantaneous Flash
Storage Conditions	2 - 8°C; Store in amber bottle to protect from light
Shelf Life	1 year
Working Solution	Ready to use solution

Ordering Information

Description	Catalog Number
Lumigen SPARCL	SDK-10K

Please visit www.LUMIGEN.com or contact LUMIGEN to request a quote.

