Lumigen SPARCL™ Detection Kit

Introduction

SPARCL™ (Spatial Proximity Analyte Reagent Capture Luminescence) is a proximity dependent, non-separation, chemiluminescent technology for the detection of specific binding interaction or association between two binding partners such as protein-protein, protein-antibody, DNA-protein, and DNA-DNA. 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. A flash of chemiluminescence is generated upon addition of a trigger solution. This assay technology is applicable to both sandwich and competitive assays. The assay can be implemented in both solid and solution formats without a wash step. A background reducing reagent is added to enhance the signal to noise ratio.

SPARCL Kit Components

Item	Quantity	Amount	Storage
Lumigen SPARCL Labeling Reagent	1 vial	0.5 mg	-35ºC to -10ºC
BupH™ Borate Buffer Pack (Thermo Scientific)	1 pack*	For 500 mL	2-8ºC
Lumigen SPARCL Background Reducing Agent	1 vial	10 mL	2-8ºC
Lumigen SPARCL Trigger Solution	1 bottles	100 mL	2-8ºC

* Dissolve the contents of the borate buffer pack in 500 mL of deionized water to make 0.05M sodium borate buffer with pH 8.5.

Reagents, Supplies and Equipment not provided

Dimethylformamide (DMF)
1X PBS
White Microtiter well Plates (Recommend Greiner or Corning Plates)
Luminometer with injectors

Lumigen SPARCL Labeling Reaction

1. Add 500 µL of DMF to Lumigen SPARCL labeling reagent vial. (The reconstituted labeling reagent is sufficient for at least ten 1 mL labeling reactions of protein with

molecular weight of 150 kDa – see below). This is a single use only vial. Do not store it and try to use it again.

2. In a separate 1.5 mL microfuge tube mix the following in this order:
   708.7 µL of 0.05M sodium borate buffer, pH 8.5
   250 uL of a 1 mg/mL antibody solution (approximately 150 kDa)
   41.3 µL of Lumigen SPARCL labeling reagent in DMF (from step 1)

Note: To label a protein with different molecular weight, use the following formula to calculate the amount of Lumigen SPARCL reagent to be added (in µL) to 1 mL reaction,

41.3 µL * (150 kDa/Protein Molecular Weight)

3. Mix by inverting the tube 4-5 times, cover the tube with aluminum foil and let stand for 30 min. at room temperature.
4. Place the labeling reaction tube on a rocker to mix at 2-8º C overnight.

Lumigen SPARCL Data Sheet

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.

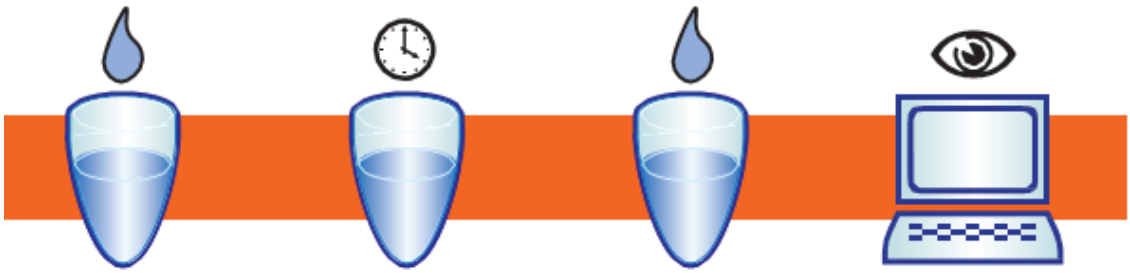
Flexible assay formats

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.

Steps – 30 minutes – No solid support – No wash



Add sample and labeled antibodies	Incubate 5–30 minutes	Add background reducing agent	Inject trigger and instantly read signal on luminometer

SPARCL vs Conventional ELISA Assays

No Wash Steps

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.

No Solid Phase

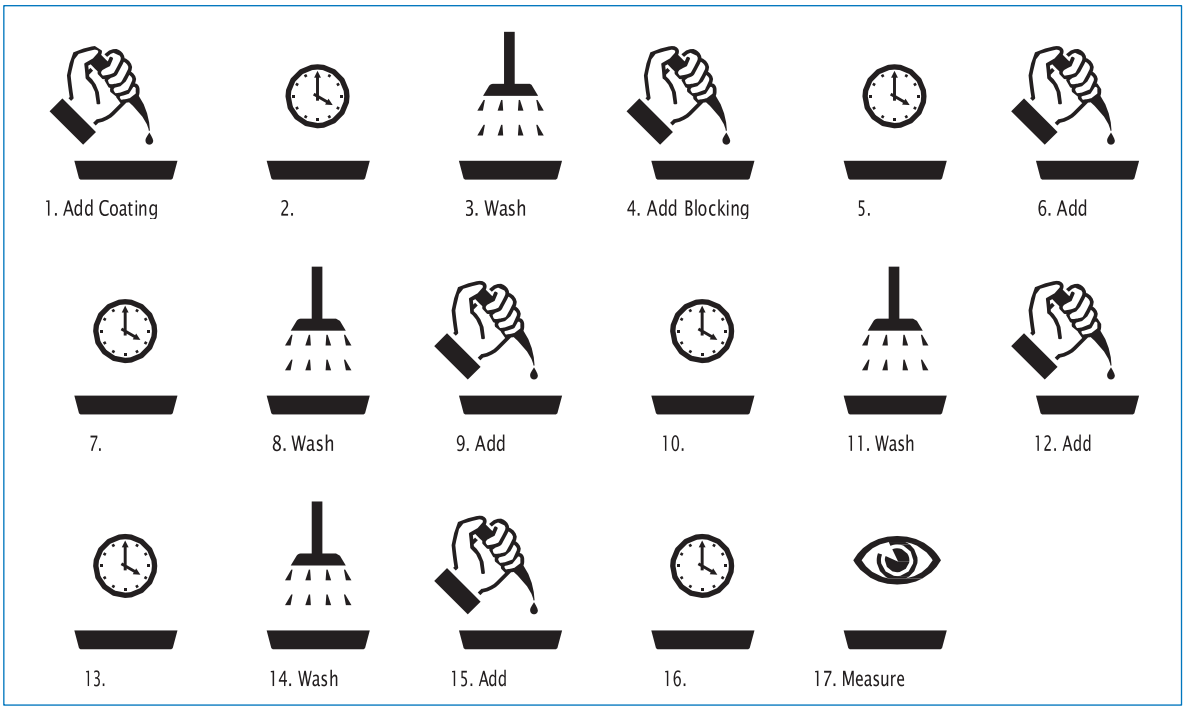
Tethering of a capture molecule to a solid support is essential for an ELISA assay as this enables the separation of specifically bound molecules from those that are not bound. Tethering is not required in a SPARCL assay.

Flash Signal Generation

Conventional ELISA assays require the substrate and enzyme to develop for up to 30 minutes before measuring signal.

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)

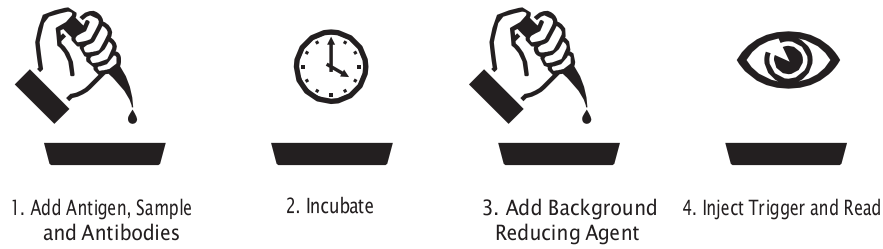


Figure 1. Comparative workflows between conventional ELISA assays and SPARCL assays.

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.

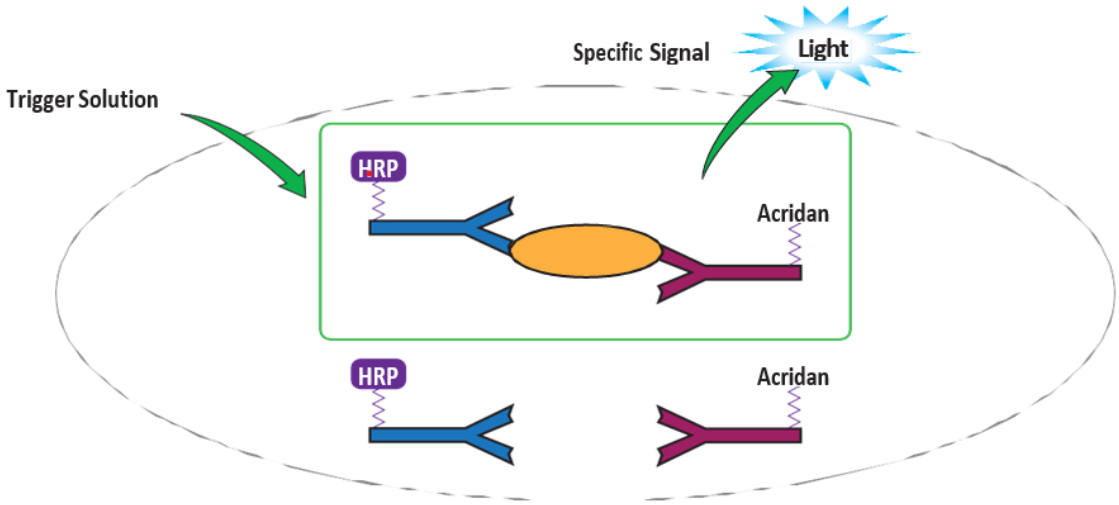


Figure 2. A Simplified Mechanistic Scheme of Spatial Proximity Analyte Reagent Capture Luminescence (SPARCL) Technology

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

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.

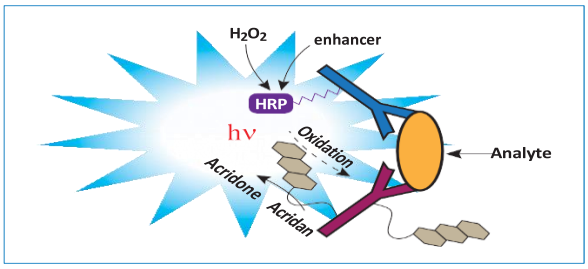


Figure 3. Sandwich Assay – Solution Phase

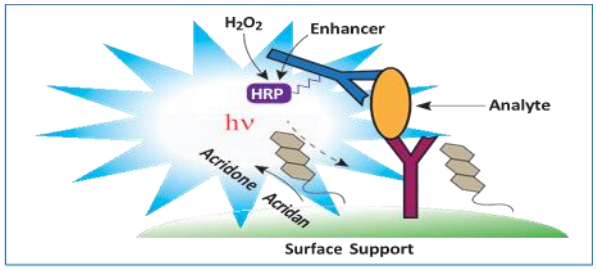


Figure 4. Sandwich Assay-Solid Phase

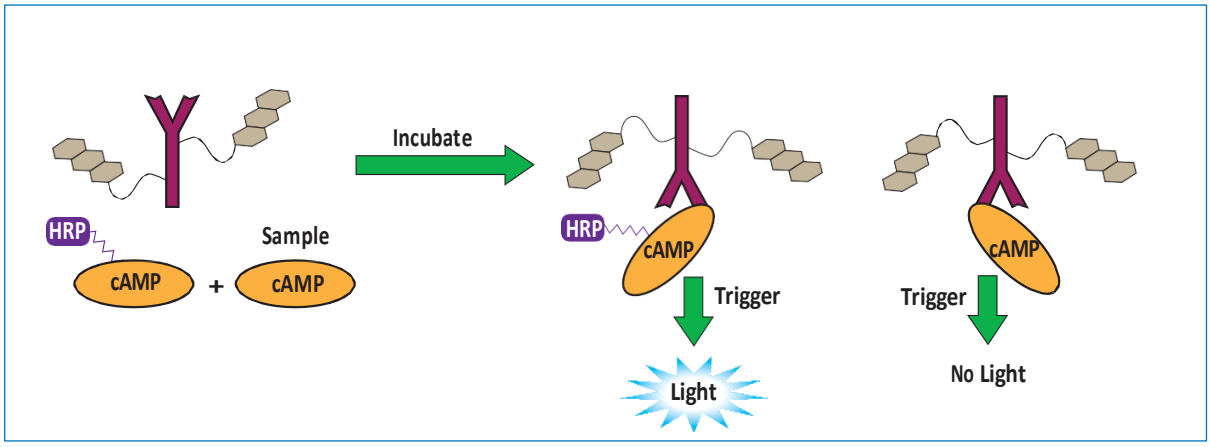


Figure 5: Competitive assay - solution phase

Product Specifications

Enzyme	Horseradish Peroxidase (HRP)
Application	ELISA, Proximity Assays
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