SPARCL™ EIA Kit

Table 1-1: Available Kit

Assay Kit	Part Number
SPARCL™ EIA Kit	R8218

The SPARCL™ EIA Kit provides a homogeneous assay using flash chemiluminescence detection without solid support, separate antibody incubations, or wash steps, allowing assays to be completed in 60 minutes or less.

Contents

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Molecular Devices

www.moleculardevices.com

Product manufactured by Molecular Devices, LLC.
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Molecular Devices, LLC is ISO 9001 registered.

Chapter 1: About the SPARCL EIA Kit

The SPARCL EIA Kit provides a homogeneous assay using flash chemiluminescence detection without solid support, separate antibody incubations, or wash steps, allowing assays to be completed in 60 minutes or less. 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.

Advantages

- Complete assays in 60 minutes with just one incubation step
- Homogenous assay requires no washing and produces less waste
- After SPARCL Labeling Reagent is conjugated to antibody or protein, further purification is not required, saving time
- Study a wide variety of targets with flexible solution or solid phase assay formats

Assay Principle

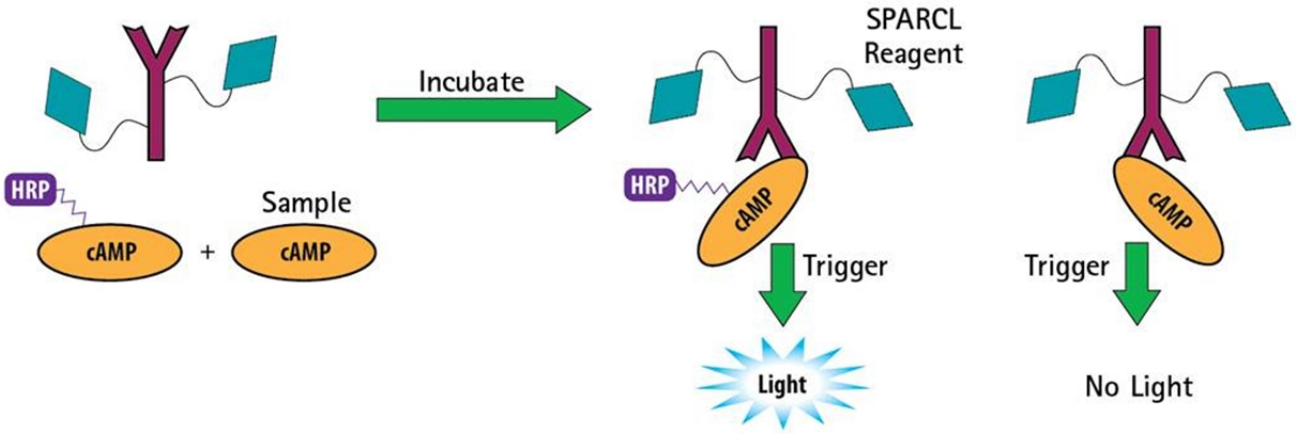


Figure 1-1: SPARCL EIA Kit competitive assay principle

In a SPARCL EIA Assay seen in the figure above, a binding partner labeled with a chemiluminescent substrate (SPARCL Labeling Reagent) 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 the SPARCL Labeling Reagent to the HRP, a flash of chemiluminescence is generated upon addition of a Trigger Solution. The presence of the special Background Reducing Agent enables the assays to be truly homogeneous and target-specific. Using SPARCL technology, assays can be miniaturized for high throughput screening while maintaining sensitive results with good dynamic range. The solution phase kinetics of the kit eliminates variability inherent in attachment to solid phase producing faster, more accurate results. Because of the simple workflow incorporating one incubation step, the SPARCL EIA Assay can be automated and adapted for multiple applications including ELISA, protein-protein and protein-nucleic acid interactions, and high-throughput binding assays.

Chapter 2: Materials and Equipment

Kit Components

Table 2-1: Components of the SPARCL EIA Kit

Item	Quantity	Part Number
SPARCL EIA Labeling Reagent, 0.5 mg	1 vial	R7571
SPARCL EIA Background Reducing Agent, 5 mL	1 bottle	R7572
SPARCL EIA Trigger Solution, 100 mL	1 bottle	R7573

The entire SPARCL EIA Kit (R8218) is sufficient to perform approximately one thousand (1000) 96-well assays or three thousand (3000) 384-well assays, depending on optimization.

Materials Required but Not Provided

- Protein or antibody of interest to be labeled, at 1 mg/mL in PBS
- HRP-Conjugated protein or antibody of interest
  These two proteins or antibodies should be a good binding pair.
- N,N-Dimethylformamide (DMF)
- 50 mM sodium borate buffer, pH 8.5
- 1X phosphate buffered saline (PBS)
- Bovine Serum Albumin (BSA)
- 96-well or 384-Well White Microplates
  Molecular Devices recommends Corning microplates.
- SpectraMax® L Luminescence Microplate Reader
- SoftMax® Pro Microplate Data Acquisition and Analysis Software, version 5.4.6

Storage and Handling

On receipt of the SPARCL EIA Kit, store the SPARCL EIA Labeling Reagent at –20°C, and store the SPARCL EIA Background Reducing Agent and SPARCL EIA Trigger Solution at 4°C to 8°C. Under these conditions the reagents are stable for **three months** in the original packaging.

WARNING! Reagents can contain chemicals that are harmful. Exercise the applicable care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site: www.moleculardevices.com/support.html

Chapter 3: Assay Protocols

SPARCL EIA Labeling Reaction

1. Add 500 µL of DMF to the SPARCL EIA Labeling Reagent vial.
2. Mix to dissolve the reagent completely.
   The reconstituted SPARCL EIA Labeling Reagent is sufficient for at least ten 1 mL labeling reactions of protein with a molecular weight of 160 kDa. See the formula in step 3.

   Note: The SPARCL EIA Labeling Reagent should be freshly prepared before the labeling reaction. After the reagent is reconstituted, use the SPARCL EIA Labeling Reagent immediately. Discard the unused portion.

3. In a 1.5 mL microfuge tube, mix the following:
   - 710 µl of 50 mM sodium borate buffer, pH 8.5
   - 40 µl of reconstituted SPARCL EIA Labeling Reagent in DMF, from step 1
   - 250 µl of 1 mg/mL protein or antibody (approximately 160 kDa)

     Note: To label a protein with a different molecular weight, use the following formula to calculate the amount of the SPARCL EIA Labeling Reagent to be added to the reaction, and adjust the buffer amount to a total of 1 mL reaction.

     Volume (µL) = 40 µL * (160 kDa/Protein Molecular Weight)

4. Mix by inverting the tube 4 to 5 times.
5. Cover the tube with aluminum foil and let it stand for 30 minutes at room temperature.
6. Store the labeled proteins at 4°C away from light for up to 3 months.

Note: No purification step is necessary.

Homogeneous SPARCL Assay

It is essential that the binding partners are of high titer and have highly specific binding. The labeled binding partners need to be titrated and tested in the SPARCL assay to determine the optimal concentrations for maximum detection sensitivity with minimal background. For example, the antibody stocks from commercial vendors can vary in their binding specificity and protein concentration. As a general rule, SPARCL EIA labeled antibodies can be diluted and used in the range of 1 to 3 µg/mL, and the HRP-conjugated antibodies in the range of 0.1 to 0.3 µg/mL.

Experiment Protocol

Note: The SPARCL assay is highly flexible and easily customized. The following protocol is only a suggestion . Each individual assay component should be optimized to achieve the best performance.

1. Make sure all buffer solutions and SPARCL EIA reagents are at room temperature, while keeping the labeled protein or antibody and HRP-conjugated protein or antibody on ice.
2. For detecting an antigen, add 15 µL (384 well) or 40 µL (96 well) of the antigen sample to each well. For detecting a protein-protein interaction, add 1X PBS containing 0.1% BSA in place of the antigen.
3. As noted in the general rule above, dilute the SPARCL EIA labeled protein/antibody and HRP-conjugated protein/antibody to the appropriate concentrations in 1X PBS containing 0.1% BSA or assay specific buffer, for example, lysis buffer for cell-based assays requiring cell lysis.
4. Add 7.5 µL each (384 well) or 20 µL each (96 well) of the diluted SPARCL EIA labeled and HRP-conjugated proteins/antibodies from step 3 to the wells containing samples from step 2.
5. Cover the microplate and incubate at room temperature for 30 to 60 minutes **in the dark**.
6. During the incubation period, set up your instrument and software protocol. See Setup for the SpectraMax® L Luminescence Microplate Reader on page 8.
7. At the end of the incubation period, add 1 µL (384-well) or 2 µL (96-well) of SPARCL EIA Background Reducing Agent to the reaction from step 5.

   Tip: For easier liquid handling, the SPARCL EIA Background Reducing Agent can be diluted 5 fold in 50 mM sodium borate buffer, pH 8.5, for an addition of 5 µL (384-well) or 10 µL (96-well) to the reaction.

8. Prime the Measurement (M) injector on the SpectraMax L Luminescence Microplate Reader with 8 mL water first and then with SPARCL EIA Trigger Solution. The 1 PMT system takes 1 to 2 mL and the 2 PMT system takes 2 to 4 mL to prime, depending upon the length of the tubing.
9. Place the plate in the SpectraMax L Luminescence Microplate Reader and start the assay using the software protocol set up in step 6.
10. Use the SoftMax Pro Software to calculate and graph RLU area under the curve during the 1 second integration time.

Setup for the SpectraMax® L Luminescence Microplate Reader

Acquire fast-kinetic luminescence data from the prepared microplate using the SpectraMax® L Luminescence Microplate Reader and the SoftMax Pro Software, version 5.4.6 from Molecular Devices with the following suggested settings.

Integration/Point Count:

- Integrate: 0.02 seconds
- Repeat: 50 times

Sensitivity:

- PMT: Photon Counting
- Correction: None

Automix:

- Off

Aperture:

- Large for 384-well and 96-well

Injection and Delay:

- P-Injection
  - Off
- M-Injection
  - M injector volume (µl): 30 (384-well) or 80 (96-well)
  - Baseline read delay (sec): 0
  - Injection speed (µL/s): 230 (384-well) or 350 (96-well)
  - Shake after injection (sec): 0
  - Number of baseline reads: 0

Injection Wells:

- M-injector: all wells selected in Wells to Read

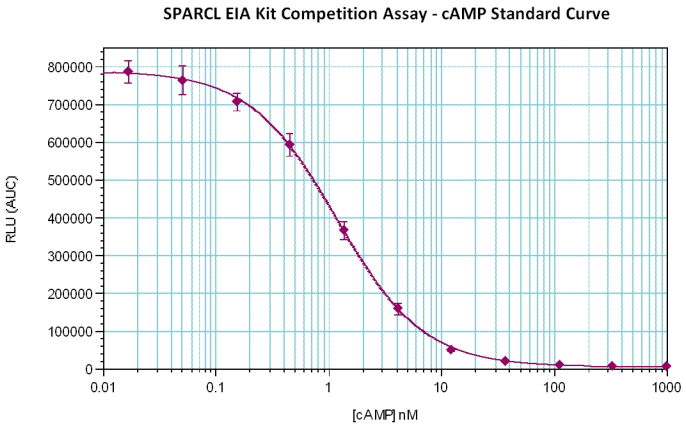
Dark Adapt:

- Delay: 0.5 to 1 minute

Chapter 4: Data Analysis Examples

SPARCL EIA Competition Assay for cAMP

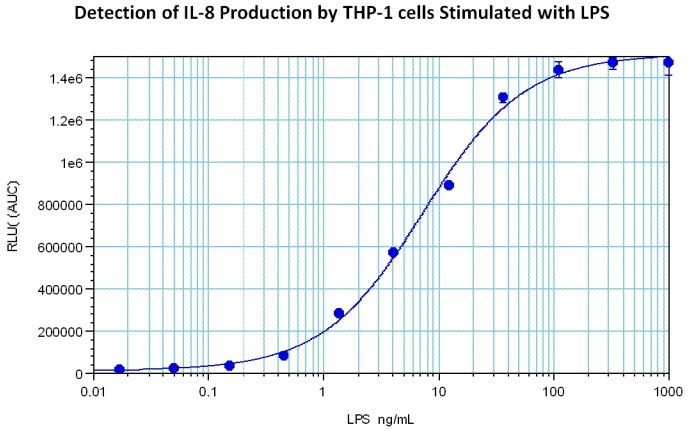
The SPARCL EIA Kit can be used in a cAMP competitive assay as shown in the figure below. cAMP antibody labeled with SPARCL reagent, cAMP conjugated Horseradish Peroxidase (HRP), and sample containing cAMP were incubated together for 60 minutes in a 384-well white plate in DPBS + 0.1% BSA. After addition of SPARCL Background Reducing Agent, the plate is ready for injection of SPARCL Trigger Solution. In a competition assay, HRP conjugated cAMP competes with sample cAMP to bind to cAMP antibody labeled with SPARCL Reagent. As seen in the figure, higher cAMP sample concentrations increase competition and decrease the SPARCL signals upon addition of Trigger Solution.



The figure above shows a cAMP standard curve in DPBS + 0.1% BSA generated in a SPARCL EIA competition assay in a 384-well format. RLU was determined as area under the curve during a total of one second read time for each well on the SpectraMax® L Luminescence Microplate Reader. In this assay, IC_{50} = 1.2 nM and Z' factor is 0.93.

IL-8 production by THP-1 cells in response to LPS

A solution phase sandwich assay was performed using the SPARCL EIA Kit to measure signal increase with increasing amounts of IL-8 produced in response to lipopolysaccharide (LPS). THP-1 cells were set up in culture at one million cells/mL and incubated for four hours at 37 °C with a half log dilution series of LPS starting at 1 µg/mL. Samples from each concentration were incubated for one hour with Anti-IL-8 antibody labeled with SPARCL and Horseradish Peroxidase (HRP) conjugated Anti-IL-8 antibody. Background Reducing Agent (BRA) was added to each well and the plate was transferred to the SpectraMax® L Luminescence Microplate Reader. The signal was measured for a total of one second immediately after SPARCL Trigger Solution was injected into each well.



The figure above shows a concentration response curve of IL-8 production by LPS stimulation of THP-1 cells. The IL-8 assay was performed using the SPARCL EIA Kit in a 96-well format. RLU area under the curve was calculated and plotted using SoftMax® Pro Software. In this assay, EC_{50} = 7.3 ng/mL and Z factor at EC_{80} = 0.94.

Chapter 5: Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, www.moleculardevices.com/support.html , has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

Please have the product name, part number, and lot number available when you call.

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