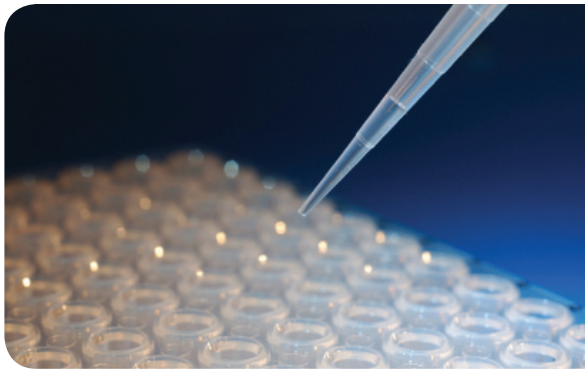
Molecular Devices

Protein quantitation with SPARCL EIA Kit



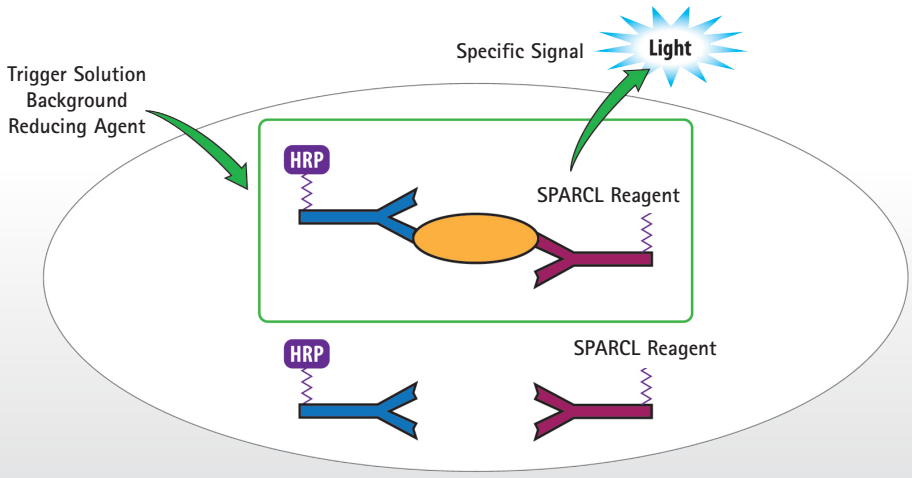
The SPARCL™ EIA kit is a homogeneous assay utilizing flash chemiluminescence detection without solid support, separate antibody incubations, or wash steps, allowing assays to be completed in 60 minutes or less. Spatial Proximity Analyte Reagent Capture Luminescence (SPARCL) is a proximity dependent chemiluminescent technology for the detection of specific binding interaction or association between two binding partners. In a SPARCL EIA assay (Figure 1), 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 enzyme in the presence of Background Reducing Agent, a flash of chemiluminescence is generated upon addition of a Trigger Solution.

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 mimic the native in vivo environment by eliminating 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 (Figure 2).

Benefits

- Complete assays in 60 minutes in a single incubation step
- Homogenous no-wash assay produces less waste
- Antibody conjugation without purification
- Flexible assay format for a wide variety of targets

Figure 1. Simplified mechanistic scheme of SPARCL EIA technology



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

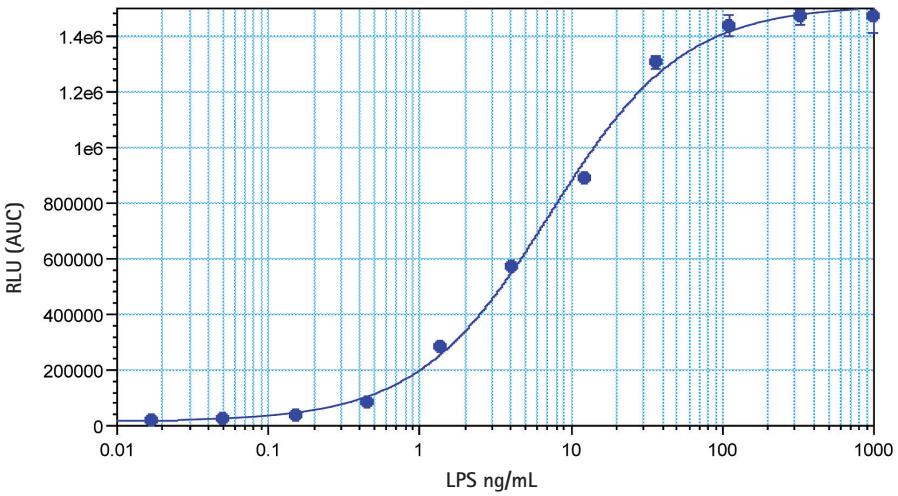
A solution phase sandwich assay was performed using the SPARCL EIA Kit to measure signal increase with increasing amounts of IL-8 produced in response to lipopolysaccharide (LPS). THP-1 cells were set up in culture at one million cells/mL and incubated for four hours at 37 °C with a half log dilution series of LPS starting at 1 mg/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 the SPARCL Trigger Solution was injected into each well (Figure 3).

SPARCL EIA competition assay for cAMP

The SPARCL EIA Assay Kit can be used in a cAMP competitive assay as shown in Figure 4. 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 Figure 5, higher cAMP sample concentrations increase competition and decrease the SPARCL signals upon addition of Trigger Solution.

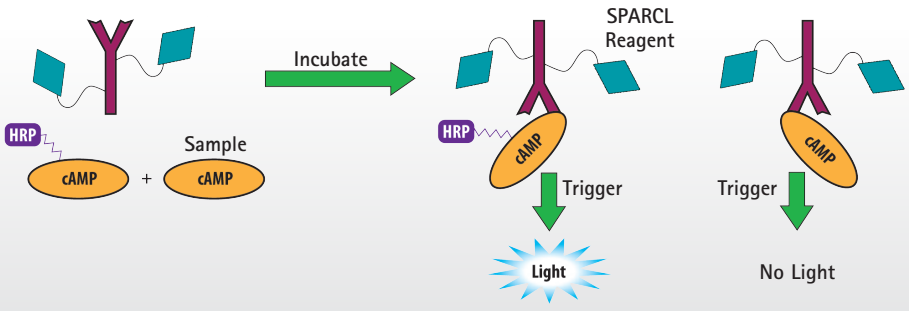
Figure 2. SPARCL EIA Kit assay workflow

Figure 3. Detection of IL-8 production by THP-1 cells stimulated with LPS



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. EC_{50} = 7.3 ng/mL and Z factor at EC_{80} = 0.93.

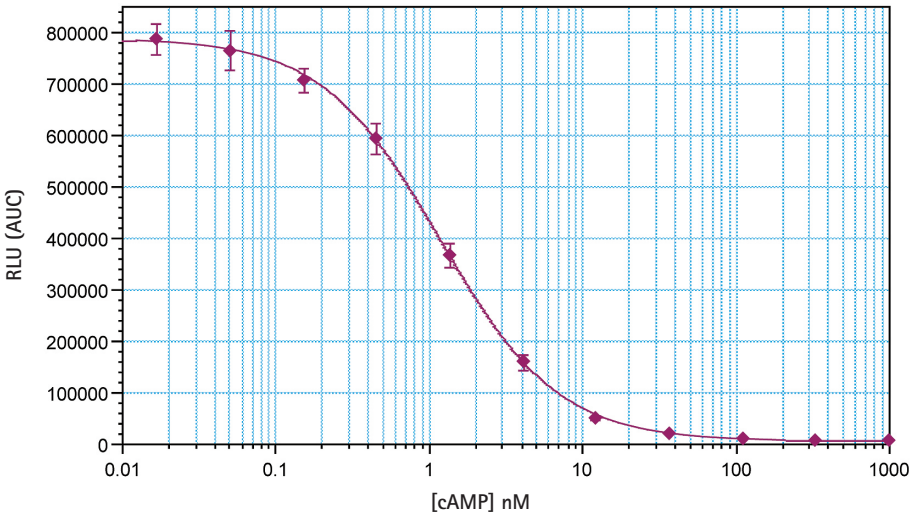
Figure 4. SPARCL EIA solution phase competition assay



Conclusion

We have shown the application of the SPARCL EIA Kit in both a solution phase sandwich assay and a competition assay. Both assays are homogenous and save time and expense as they do not require separate incubations, blocking or wash steps. The SPARCL EIA Kit is a fast, homogenous and flexible assay platform adaptable to multiple applications including ELISA, protein-protein interactions, protein-nucleic acid interactions, and high throughput binding assays.

Figure 5. SPARCL EIA Kit competition assay – cAMP standard curve



A cAMP standard curve in DPBS + 0.1% BSA was 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.

Ordering Information

Reagent	Description	Part Number
SPARCL EIA Kit	SPARCL EIA Labeling Reagent SPARCL EIA Background Reducing Agent SPARCL EIA Trigger Solution	R8218

Compatible with this Molecular Devices® system

SpectraMax® L Luminescence Microplate Reader

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