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SPARCL

A 'No-Wash' Cost-Effective High-Throughput Screening Alternative for Detecting Binding Interactions

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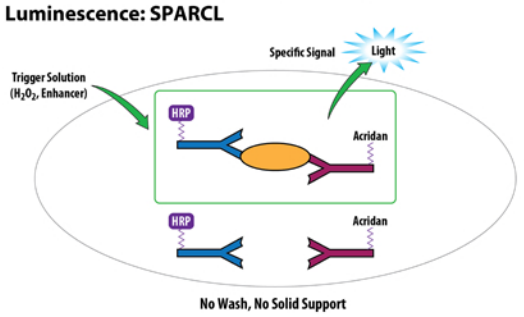
Abstract

Biomolecular binding interactions are commonly monitored using antibodies and other proteins with detectable labels in formats such as ELISA and close proximity assays. SPARCL is a no-wash, cost effective and flexible proximity assay capable of detecting common protein targets, as well as targets that are difficult to measure using other homogeneous assay technologies. SPARCL technology allows for simple reagent preparation and rapid assay optimization without dependence on particles or solid surface.

Examples of antibody-antigen assays (e.g. cAMP, GM-CSF) as well as protein-protein interactions are shown in a variety of formats demonstrating the flexibility of the SPARCL system. The application of SPARCL technology to a high-throughput environment and technology flexibility is shown in these assays.

Spatial Proximity Analyte Reagent Capture Luminescence: SPARCL



Sandwich Immunoassays

Traditional ELISA style binding assays involve an antibody pair and a solid substrate. For these assays a SPARCL detection kit was combined with commercially available antibodies and calibrators.

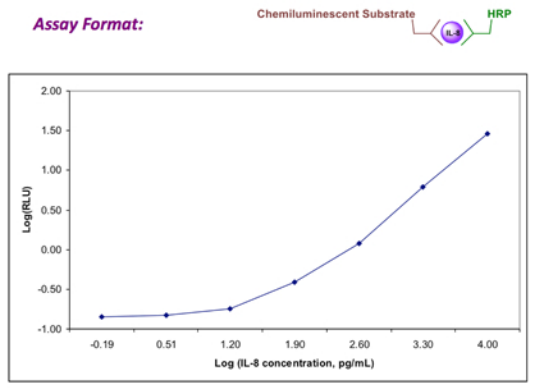
Step 1: Add the activated SPARCL label to the capture protein. Label corresponding detection protein with HRP. No wash or purification required.

Step 2: Add 10 µL of each labeled protein to 10 µL of sample. Incubate 30 minutes

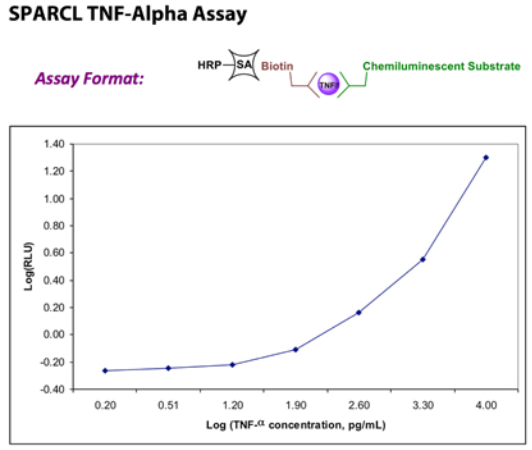
Step 3: Add 1 µL SPARCL Background Reducing Agent

Step 4: Collect results, using a plate reading luminometer, injecting 30 µl SPARCL trigger solution.

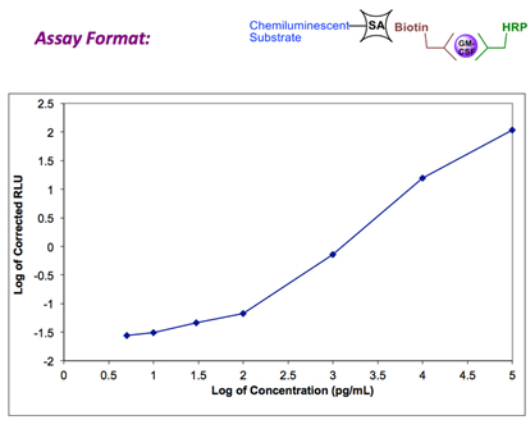
SPARCL IL-8 Assay



SPARCL TNF-Alpha Assay



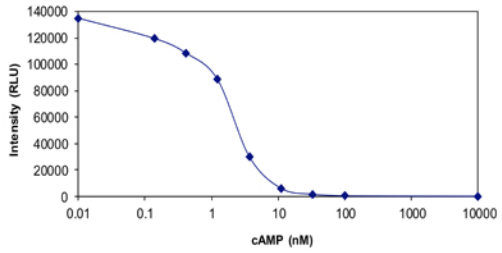
SPARCL GM-CSF Assay in Serum



IL-8, TNF-Alpha from R&D Systems. GM-CSF from Leinco.

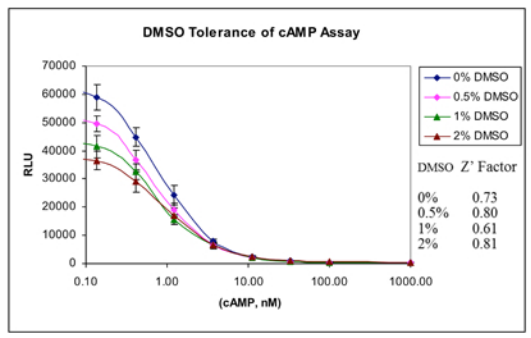
Competitive Immunoassays

cAMP



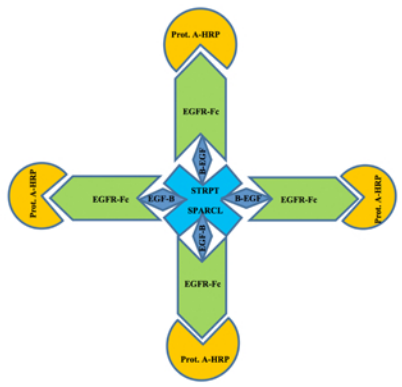
For this popular research target, the SPARCL assay was miniaturized using 3 µL Anti-cAMP antibody (Immuotech) conjugated the chemiluminescent label. This was incubated with 3 µL of cAMP-HRP conjugate for 15 minutes. 4 µL of each unknown sample was added; after 30 minutes of incubation 1 µl of SPARCL Background Reducing Reagent was added and the well intensities were measured with 10 µL of SPARCL Trigger Solution.

cAMP with DMSO



Varying levels of DMSO were added to simulate needs of a high-throughput environment. The Z Prime values indicate that the SPARLC cAMP assay maintains its sensitivity and usefulness.

Protein Interaction SPARCL Scheme



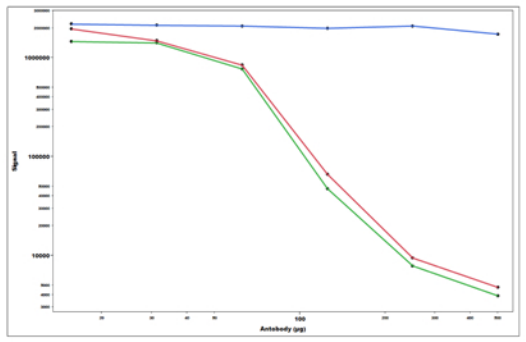
Protein Interaction SPARCL Protocol

1. EGF-biotin and EGFR-Fc proteins are bound to each other for 60 min. in 50 µL of 1X TBS containing 0.5% BSA

2. Then 25 µL each of acridan labeled streptavidin (Streptavidin-SPARCL) and Protein A-HRP diluted in 1XTBS/0.5% BSA were added and incubated for 30 min.

3. Each 100 µL reaction was split into 50 µL in duplicate white microtiter wells (Nunc, Denmark) and a background reducing reagent was added to minimize noise due to free streptavidin-SPARCL and protein A-HRP that were not bound to the EGF/EGFR protein pair.

4. To read the chemiluminescent SPARCL signal, 50 µL of trigger solution was injected into each well on SpectraMax (Molecular Devices, Sunnyvale, CA).



The red line represents Anti-EGFR prebound to the complex before the HRP conjugate is added. A decrease in signal demonstrating that SPARCL accurately tracks the blocking of the complex. Similar results were observed adding Ant—EGFR after the binding was completed (green line). The blue line is a nonspecific antibody being added to show that results are indicative of the specific interaction.

Conclusions

- Homogeneous SPARCL assays can detect targets in a variety of formats without a solid support and washing steps
- Because of the fewer steps and ease of detection, the homogeneous SPARCL can be automated for high throughput detection of protein-protein interactions and for screening of protein interaction inhibitors
- Special antibodies and sophisticated/custom preparation techniques are not required.
- The assay reagent volumes can be reduced to fit in 384 well plates
- Some of the distinct advantages of homogeneous SPARCL are that there are no beads or particles, fast read time due to flash of signal and requires only a luminometer with an injector.