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Fluorescent Assay for Determining Phosphoribosyl Ubiquitination

Real-time fluorescence polarization assay for SidE-catalyzed ubiquitination enables high-throughput discovery of Legionella infection inhibitors.

Purdue University researchers have developed a fluorometric assay for real-time monitoring of ubiquitination events catalyzed by bacterial SidE enzymes in Legionella infections. The assay is well suited for high-throughput identification of Legionella infection inhibitors. The enzymes of the Legionella SidE family catalyze host protein ubiquitination events via a recently discovered mechanism distinct from ubiquitination by eukaryotic enzymes and are required for optimal Legionella infection. Contemporary methods to investigate the ubiquitination activity of SidEs include mass spectrometry and SDS-PAGE gel shift assays. These current techniques are not continuous, only measuring the end point of the reaction, and are not amenable to high throughput formats. Purdue researchers synthesized a fluorescently labelled synthetic substrate peptide for SdeA, a member of the SidE family, that displays a change in fluorescence polarization when ubiquitinated by the enzyme. This technology is amendable to high throughput screening and will assist in discovery of inhibitors for Legionella infection as well as identifying and characterizing SidE-like enzymes in other bacterial species.

Advantages:

-Real-Time SidE Ubiquitination Analysis

-Amenable to High Throughput Screening

Potential Applications:

-Legionella Research

-Investigating Ubiquitination Events

-Fluorescence Polarization

Related Publication:

Technology ID

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Category

Biotechnology & Life Sciences/Biomarker Discovery & Diagnostics

Pharmaceuticals/Drug Discovery & Development

Biotechnology & Life Sciences/Analytical & Diagnostic Instrumentation

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Fluorescent Probes for Monitoring Serine Ubiquitination

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