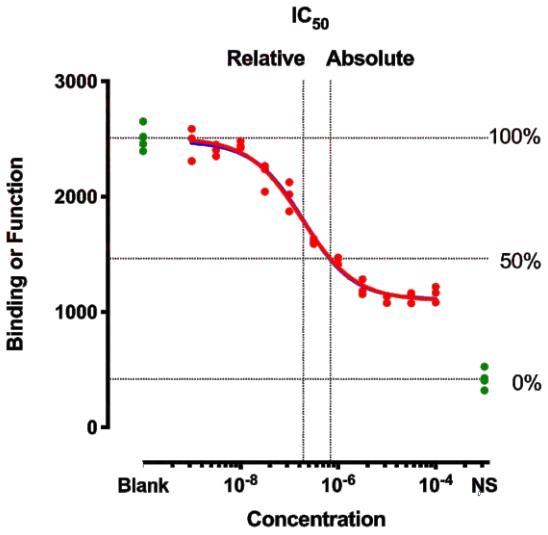
Myra Liquid Handling System

Kinase Assays

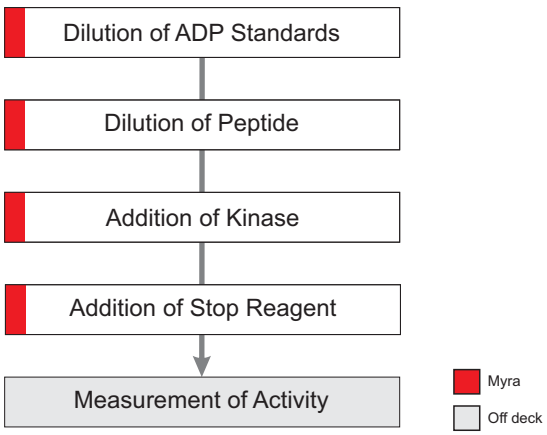
Myra for Kinase Assays

Accurate measurement of kinase activity depends on the meticulous addition of reagents, substrates, and inhibitors at specific concentrations, volumes, and times. Myra has shown to provide unmatched precision in liquid dispensing, reduced manual intervention, and minimized human errors, revolutionizing the conduct of kinase assays.



Key Features

Myra can be programmed to handle complex liquid dispensing routines, including serial dilutions for creating concentration gradients of substrates or inhibitors, crucial for kinetic analysis and inhibitor characterization. The ability to execute these routines quickly and with high precision at volumes as small as 1 µL, directly impacts the quality and reliability of the data collected.



Types of Kinase Assay Run on Myra

- The ADP-Glo Kinase Assay is a luminescent assay that measures the amount of adenosine diphosphate (ADP) produced by kinase reactions over time.
- Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is a sensitive and reliable fluorescence-based assay technique that combines the principles of time-resolved fluorescence and FRET to minimize background signal and maximize signal-to-noise ratio.

Advantage of Myra for Kinase Assays

Myra facilitates the generation of data sets necessary for enzyme kinetic analysis by ensuring that reaction conditions are consistently replicated, evan at volumes as low as 1 µL. This uniformity allows for the precise determination of reaction velocities at varying substrate or ligand concentrations, critical for plotting kinetic assay curves and calculating kinetic parameters.

Myra enables high-throughput screening of kinase inhibitors by allowing rapid and consistent testing of numerous compounds across a range of concentrations. This capability is invaluable in the early stages of drug discovery, where the efficiency of screening can significantly impact the speed at which potential therapeutic candidates are identified.

Summary

Myra's sophistication and capability has made it an essential instrument for kinase assay protocols and the kinetic analysis workflow. Myra's role in advancing drug discovery by enabling more accurate, efficient, and high-throughput screening of kinase inhibitors cannot be overstated. For researchers in the field, the adoption of Myra represents a strategic investment in the quality and effectiveness of their experimental workflows.

Case Study: Promega ADP-Glo Kinase.

An IC_{50} dose-response was run to determine the potency of two CHK1 inhibitors using ADP-Glo™ Kinase assay.

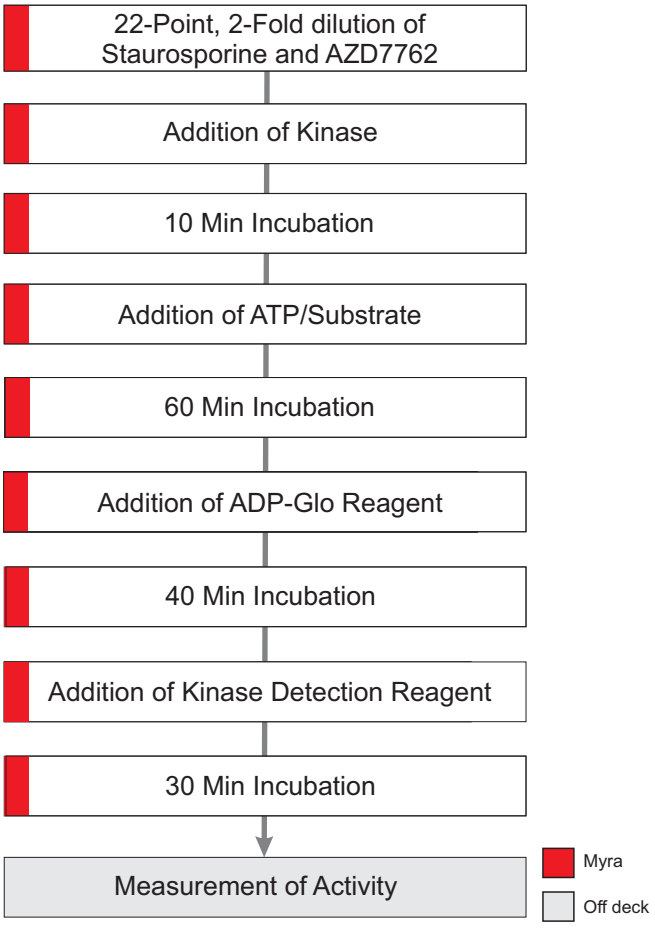
Myra Kinase Assay Protocol

In a typical ADP-Glo™ Kinase Assay, the process starts with the kinase reaction, where a kinase enzyme phosphorylates a substrate in the presence of ATP. The reaction is halted by adding ADP-Glo™ Reagent, which depletes remaining ATP and enables selective detection of ADP. The Kinase Detection Reagent then converts ADP back to ATP, generating a luminescent signal proportional to kinase activity. When performed across a dilution series of inhibitors, this allows for dose–response analysis to assess inhibitor potency.

Automating this sequence with Myra ensured that each step was precisely timed and executed, maintaining the integrity and consistency of the assay across multiple wells and plates using very small volumes (1 - 2 µL).

Experimental Setup

CHK1 kinase activity was assessed using an ADP-Glo kinase assay, following a 1 µL treatment with a 22-point, 2-fold serial dilution of Staurosporine or AZD7762, run in duplicate. Reactions contained 2 µL of 4 ng CHK1, 2 µL of 50 µM ATP/0.2 µg/µL CHKtide Substrate and were incubated for 60 minutes at room temperature. Reactions were carried out on a 384-well flat-bottomed microplate.



Precise timers were integrated into the script for the systematic addition of Stop and Detection reagents ensuring kinetic consistency across the plate. Fluorescence was read on a Hidex plate reader.

Results

Relative luminescence units (RLU) were plotted against log10-transformed inhibitor concentrations (nM). A four-parameter logistic (4-PL) non-linear regression provided IC_{50} estimates for Staurosporine and AZD7762.

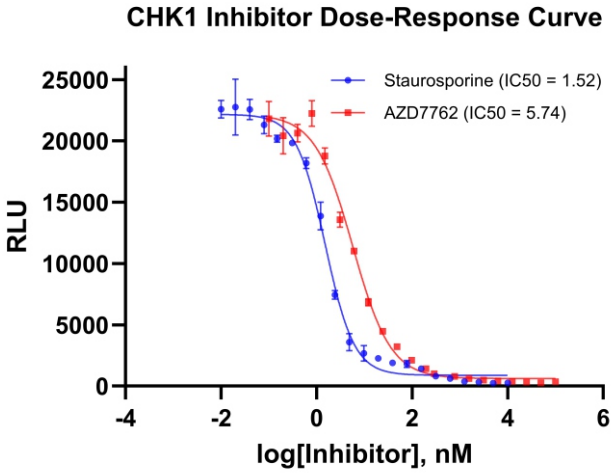


Figure 1.CHK1 kinase activity was assessed using the CHK1 Kinase Enzyme System in combination with ADP-Glo, following treatment with a 22-point, 2-fold serial dilution of Staurosporine (blue circles) and AZD7762 (red squares). Data represent the mean ± standard deviation of technical replicates.

Staurosporine and AZD7762 displayed distinct potencies, with IC_{50} values of 1.52 nM and 5.74 nM respectively, indicating that AZD7762 is approximately 3.8 times less potent under identical assay conditions.

The resulting dose–response curves showed excellent fit ($R^2 > 0.99$), and all values fell within 0.5 nM of published IC_{50} data, validating both assay fidelity and Myra's pipetting precision.

Technical replicate variability remained below 8 % across the entire concentration range, demonstrating the reliability of automated pipetting even at such low volumes.

Conclusion

By combining Myra's precise liquid-handling with ADP-Glo™ chemistry, we generated high-quality dose-response data for CHK1 within a single microplate. The platform's accuracy at low volumes and flexibility helps streamline your kinase assay workflow.

3 Apr 2024

International
sales@biomolecularsystems.com
www.biomolecularsystems.com

UK
uksales@biomolecularsystems.com

USA
usasales@biomolecularsystems.com