Novel Gyrasol Sensor Assay Platform with the Hybrid Technology™ Multi-Mode Reader Synergy™ 4

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Abstract

The Gyrasol Sensor is a novel platform for biochemical kinase / phosphatase assays suitable for small molecule drug discovery. This homogeneous, antibody-free method is universal in nature and is based on fluorescence quench, characterized by signal increase with kinase inhibition. The assay is tolerant to wide concentrations of ATP and substrate, which makes the assay tunable for the inhibition of kinase through either substrate or ATP binding sites and adaptable to either endpoint or kinetic studies. Representative data will be provided for both kinase and phosphatase screening including PI3-K and Sphingosine, noted for their difficult lipid-based substrates. Robust assays, with z'-factors in excess of 0.8 were obtained with the Synergy™ 4 Multi-Mode Microplate Reader using a number of different fluorophores and monochromator settings.

Novel Gyrasol Sensor Assay Platform Overview

- Enzyme activity causes quench of substrate fluorescence
  - TAMRA commonly used
- In presence of inhibitors, signal increase assay

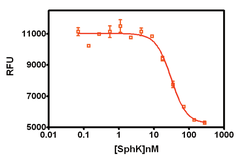
Synergy™ 4 Multi-Mode Reader with *Hybrid Technology*™

Sphingosine Kinase

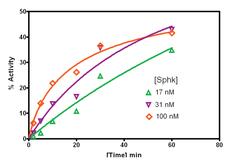
Sphingosine kinase(1) Methods

[Enzyme]: 70 nM (except in titration)
[Substrate]: 3 µM
[ATP]: 100 µM (+K_m)
Assay Buffer: Recommended Gyrasol Assay Buffer + 5 mM DTT

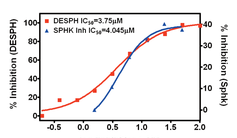
Sphingosine kinase(1) Titration



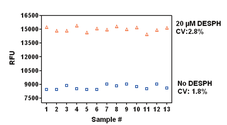
Sphingosine kinase(1) progress curve



Sphingosine kinase(1) Inhibition dose response curves



Sphingosine kinase(1) Statistics



Sphingosine kinase(1) Conclusions

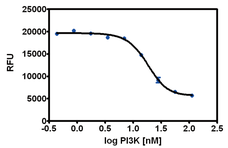
1. Sensitive Assays - LOD Sphigosine kinase 7.3 nM
2. Determined IC_{50}'s consistent with literature
3. Assay Performance suitable for screening applications
   a) z': 0.76
   b) CV's < 5%

PI-3 Kinase

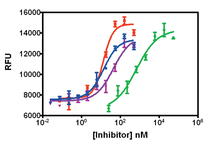
PI-3 Kinase Methods

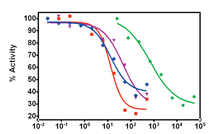
[Substrate]: 5 µM
[ATP]: 150 µM (+K_m)
Assay Buffer: Recommended Gyrasol Assay Buffer + 5 mM DTT

PI-3 Kinase Titration



PI-3 Kinase





	Gyrasol IC50 (nM)	Reported IC50 (nM)
Wortmannin	13	10
PI-103	45	8
PI-3Ka Inhibitor 2	13	2
LY294002	680	1400

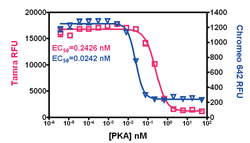
PI-3 Kinase Conclusions

1. Sensitive Assays - LOD PI-3 Kinase 1 nM
2. Determined IC_{50}'s consistent with literature

PKA - New Platform

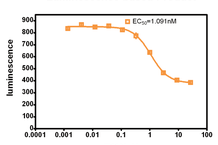
New Gyrasol Platform Developments

- Replacement of TAMRA substrate label with Chromeo 642 dye (Active Motif, San Diego)
- Forms complex with the phosphate-associated quencher, thus generating a more favorable distance for electron transfer
- Results in a more sensitive detection

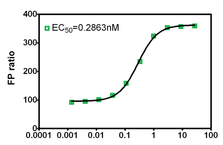


PKA Competitor Comparison with PKA

Luminescence-based Product



FP-Based Product



	LOD (nM)
TAMRA-based Gyrasol	0.2
Chromeo 642-based Gyrasol	0.002
Luminescence-based Product	0.12
FP-based Product	0.005

- Chromeo 642-based Gyrasol is most sensitive