ADP-Glo™ Kinase Profiling Systems for Targeted and Flexible Kinase Inhibitor Profiling

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1. Abstract

Drug safety is of paramount importance in the pharmaceutical industry indicating that minimal side effects constitute a major requirement in drug development. Therefore, novel drug candidates need to be profiled against various liability targets, including a broad panel of kinases to provide a better understanding of off-target activities. Potentially, profiling can also identify new targets that may lead to novel therapeutic indications. A universal, robust and affordable technology is desirable to assess selectivity and potency of drug candidates against multiple classes of kinases. The luminescent ADP-Glo™ kinase assay is a universal platform that measures kinase activity by quantifying the amount of ADP produced during the enzymatic reaction. We have tested the utility of this platform with 174 optimized Kinase Enzyme Systems (KES) spanning different families of the human kinome. Here we present standardized Kinase Profiling Systems for simple kinase inhibitor profiling studies. The Kinase Profiling Systems are a set of kinases organized by families and present in easy to use multi-well strips. Each strip contains eight enzymes each with their corresponding substrates and standardized for optimal kinase activity for inhibitor profiling. Using the profiling strips we easily generated selectivity profiles, identifying compound promiscuity towards members of a single kinase subfamily or different subfamilies of the kinome. The ADP-Glo™ KES platforms now address the needs of basic kinase characterization, kinase screening, mode of action (MOA) studies and profiling in an affordable manner using one assay format.

2. ADP-Glo™ is a positive detection assay for product formation

Assay concept, formats and Features

- Universal: Any kinase-substrate combination.
- Wide dynamic range: High sensitivity at low % ATP to ADP conversion allows use of lower amount of enzyme.
- Broad range of [ATP]: (µM to mM) allows distinction between ATP competitive and non competitive inhibitors.

3. Promega validated kinase panel covers the human kinome

Kinase Enzyme System (KES) is a complete Kinase assay solution

ADP-Glo™ Kinase Assay

- 5 mM ATP
- 100mM UltraPure ATP
- 500nM ADP-Glo Reagent
- 50mM 

Kinase Enzyme System (KES)

- AKT1 Kinase Enzyme System
- PAF1 Kinase Enzyme System
- PLK1 Kinase Enzyme System
- MEK1,2,5 Kinase Enzyme System
- MALT1 Kinase Enzyme System
- MAPKAPK2 Kinase Enzyme System
- CK1g1 Kinase Enzyme System

Broad Human Kinome coverage with >170 KES

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4. Simple protocol for flexible and targeted inhibitor profiling

Important kinase targets organized in multi-well strip panels

- Family based strips
- General panel

Streamlined profiling protocol

- Reaction Quiller
- ATP + Substrates
- ADP

5. Kinase strips make profiling with ADP-Glo™ platform simple

- Single dose Profiling
- Dose response Profiling

- Selectivity
- Promiscuity

- Enabling flexible Kinase Profiling with the Strip Systems.
- Dose response or single dose profiling against 8 kinases at once.

6. Creating selectivity profiles of inhibitors with ADP-Glo™ Kinase Profiling platform

Inhibitor profiling performed on a large scale using a single dose compound profiling protocol

ADP-Glo™ profiling systems

Data generated with ADP-Glo™ platform consistent with published potencies of radioactivity-based Kinome profiling.

Confirming promiscuity of PF-477736® compound using the dose response profiling protocol

7. Conclusion

Kinase Profiling Strip Systems have the following advantages:

- Fast and simple reaction assembly: Two quick dilutions provide working stocks of kinase and substrate/co-factor solutions sufficient for 25 kinase reactions.
- One-time use design: Eliminating multiple freeze/thaw cycles ensures optimal kinase activity for each experiment.
- Optimized kinase activity for inhibitor profiling: All kinases have been optimized to provide <35% ADP production with >10 fold S/B.
- Formatted strips provide access to eight kinases at a time: Kinases from singular kinase families are grouped together for more relevant selectivity profiles.
- Flexible kinase inhibitor profiling: Each strip has enough material to profile 4 compounds at a single dose or create a dose response for 1 compound against 8 kinases at once.