Flexible and targeted inhibitor profiling using the luminescent ADP-Glo™ kinase assay platform

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1. Abstract
Profiling kinase inhibitors is a critical step during drug development. Knowing the inhibitory profile of a compound across a broad collection of kinases can be important for better understanding its biological activity, avoiding off-target activities, and in some cases identifying new targets may lead to novel therapeutic applications. Unlike an HTS phase, where a target-specific kinase assay is used to identify hit compounds, during profiling a robust universal assay is needed to assess the selectivity and potency of inhibitor on multiple kinases from different classes. Often, these kinases use substrates with different chemical structures and target-specific screening assays are not suitable or can be cost prohibitive. We previously introduced the luminescent ADP-Glo™ kinase assay that measures kinase activity by quantifying the amount of ADP produced during the enzymatic reaction. This technology addressed all the needs of kinase screening, mode of action (MoA) studies and profiling using one assay format during the drug discovery process. Here, we show that we can achieve the sensitivity and robustness required for profiling the different kinase families covering the human kinome with one platform. The ADP-Glo™ assay was previously validated with more than hundred kinases and is now optimized for use with a large panel of complete Kinase Enzyme Systems (KES) that span different families of the human kinome. We also demonstrate the profiling of different kinase inhibitors using newly designed kinase strips. Each multiwell strip contains a group of kinases from the same family. The kinase stock volumes were standardized in a way that, when all the kinases were diluted into the kinase reaction, the kinases generated 5-10% ATP to ADP conversion. The substrate stocks were standardized in similar fashion and are located in a second strip at corresponding positions. We show that using the new kinase profiling strips and an optimized protocol, we could easily generate selectivity profiles using small or large kinase panels, as well as detecting compound promiscuity towards members of a single kinase subfamily or different subfamilies of the kinome. The fact that ADP-Glo™ platform offers so many positive attributes makes it an ideal assay not only for primary and secondary screening but also for profiling compounds in a cost-effective manner using one single platform.

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

- Step 1: Depilation of unconsumed ATP after the kinase reaction.
- Step 2: ADP is converted into ATP that is detected via a luciferase/luciferin reaction.
- Luminescent signal is proportional to ADP produced and the kinase activity.

3. ADP-Glo™ can be used with a broad range of ATP concentrations

ADF detection up to 1mM ATP in the reaction

More sensitivity with Promega new ultrapure ATP compared to other ATP sources

4. ADP-Glo™ is a universal assay validated with large kinase panel spanning the kinome

ADP-Gl™ Kinase Assay platform: Universal Kinase Assay & 150+ KES®

5. Profiling kinase inhibitors with ADP-Glo™ kinase assay

Staurosporine selectivity profiles of small kinase family panels

“One Assay for all”. ADP-Glo™ is ideal for profiling inhibitors against large or small kinase panels

6. Kinase strips for flexible and targeted inhibitor profiling

Important kinase targets organized in strips by kinase families

Streamlined profiling protocol

7. Determination of inhibitor profiles of different kinase families with ADP-Glo™ platform

Larger kinase panel for easy profiling and selectivity assays

8. General features of ADP-Glo™ platform

➤High sensitivity assay: less than 1% ATP conversion detected with more than 2.5 signal to background ratio with new Ultra Pure ATP
➤High dynamic range: High signal to background at low % ATP to ADP conversion allows use of lower amount of enzyme during HTS or profiling
➤Homogenous, non radioactive and antibody Free
➤Robust assay (Z' higher than 0.7)
➤Universal assay: Any ADP-producing enzyme (Kinase or ATPase). Ideal for inhibitor profiling with easy protocol.
➤Larger kinase panel: Complete Kinase Enzyme Systems for easy profiling and selectivity assays
➤Convenient profiling concept: Strips can be used in a mix and match fashion to profile representatives of a single kinase family or multiple families with the same assay platform

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