Kinase Strips for Routine Creation of Inhibitor Selectivity Profiles: a Novel Approach to Targeted and Flexible Profiling

Hicham Zegzouti, Jacquelyn Hennek, Juliano Alves, and Said Goueli
Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711

1. Introduction

In order to profile compounds against a broad panel of kinases it is highly desirable to have a technology that is universal, robust and affordable for everyday assessment of selectivity and potency of inhibitors against multiple classes of kinases. Although several platforms exist, most suffer from limitations that hinder their use in routine operations of kinase profiling. These include the confidentiality issues related to outsourcing the profiling, immediate accessibility to the data generated, and in house profiling can be costly and time consuming because of the assay development required for each kinase in the panel. Towards this goal, we created new kinase profiling systems for simple kinase inhibitor profiling studies based on the luminescent ADP-Glo® kinase assay platform. The kinase profiling systems have the following features and advantages:

- Set of kinases organized by kinase families, presented in easy to use multi-well strips, and standardized for optimal kinase activity.
- The strip system provides flexible kinase inhibitor profiling, as each strip can be used to profile compounds at a single dose or used for a dose response against 8 kinases at once.
- Easily automated with fast and simple reaction assembly.

Using the new kinase profiling strips we could easily generate selectivity profiles using small or large kinase panels, as well as identifying compound promiscuity towards members of a single kinase subfamily or different subfamilies of the kinome. With these advantages, the kinase selectivity chip platform can now allow for routine performance of inhibitor profiles in quick, affordable and flexible manner using one assay format for all classes of kinases during the drug discovery process.

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. Validated Kinase Panel Covers the Human Kinome

Broad Human Kinome coverage with >170 Protein Kinase Enzyme Systems (KES) and Lipid Kinase Class I

4. Kinase Strips Make Profiling with ADP-Glo® Platform Simple

Small panel inhibitor profiles can be created with KES and Lipid kinases
- Profiling requires assay development for each kinase in the panel.
- Can be time consuming and not cost effective

5. Simultaneous Profiling Compounds Against Protein and Lipid Kinases

- Profiling kinases in strips to identify compounds’ selectivity towards members of PI3K family.
- Simultaneous Profiling of Protein and Lipid kinases identifies promiscuity of PI3K purportedly specific inhibitor towards other protein kinases such as CK2a1.
- Profiling Strips easily identifies the rank order of compounds’ potencies.

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

Data generated with ADP-Glo® platform consistent with published potencies of radioactivity-based Kinase profiling.

7. Conclusions

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 optimal 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 10 compounds at a single dose or create a dose response for 1 compound against 8 kinases at once.
- Lipid kinases can be assembled in strip format to be used with the protein kinases in the same compound profiling experiments.

Corresponding author: hicham.zegzouti@promega.com

www.promega.com

February 2015