



ADP-Glo™ Kinase Profiling Application Notes

AGC KINASE KSPS: AGC-2

Kinase Selectivity Profiling System: AGC-2

By Jacquelyn Hennek, M.S., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

Kinase Selectivity Profiling System AGC-2 is a set of kinases from the AGC Kinase Family presented in an easy to use 8-tube strip format. When diluted, the kinase stock volumes are standardized to generate optimal ATP to ADP conversion with a signal to background ratio over 10-fold when their activities are detected using the ADP-Glo™ Kinase Assay (Fig. 1). The substrate stocks are standardized in a similar fashion and are located in a second strip at corresponding positions. Kinase Selectivity Profiling Systems can be used to generate single-dose inhibitor selectivity profiles for as many inhibitors as desired (Fig. 2A) or to study dose response curves for an inhibitor (Fig. 2B).

AGC-2		
AGC Family		
	Kinase Strip	Substrate Strip
A	PKC α	CREBtide + Lipid Activator
B	PKC β II	CREBtide + Lipid Activator
C	PKC δ	CREBtide + Lipid Activator
D	PKC ϵ	PKCtide + Lipid Activator
E	PKC γ	PKCtide + Lipid Activator
F	PKC ι	CREBtide + Lipid Activator
G	PKC θ	PKCtide + Lipid Activator
H	PKC ζ	CREBtide

ADP-Glo™ Kinase Assay

Description

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase.

The luminescent signal positively correlates with ADP amount and kinase activity. The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 2).

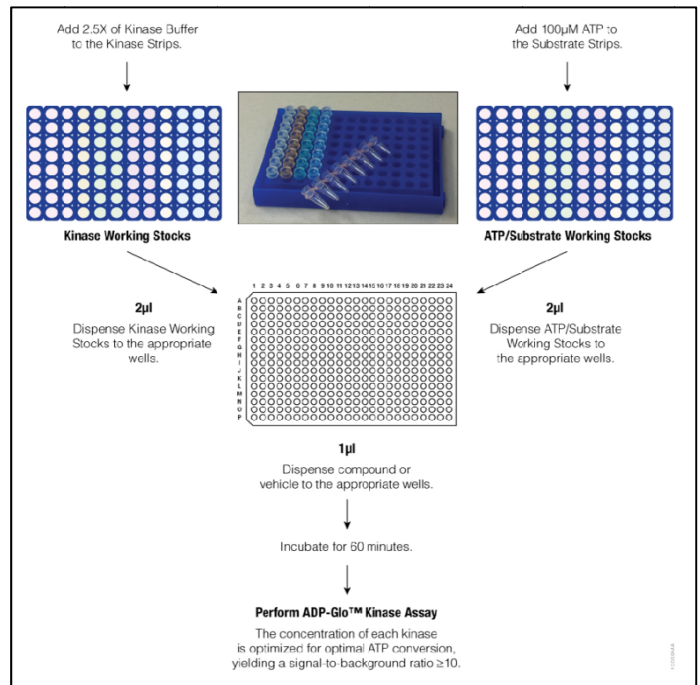


Figure 1. Kinase Selectivity Profiling System Overview. Kinases are provided at either 25X or 50X concentrations in an 8-tube strip, and substrates/cofactors are provided at 3.3X concentrations in a separate 8-tube strip. One-step dilutions directly into the strips produce sufficient Kinase and ATP/Substrate Working Stocks for 25 kinase reactions. Kinase reactions are performed using 1µL of compound, 2µL of Kinase Working Stock, and 2µL of ATP/Substrate Working Stock. After 1 hour incubation at room temperature, kinase activity is quantified using the ADP-Glo™ Kinase Assay. The luminescent signal generated by the ADP-Glo™ Kinase Assay is proportional to ADP concentration and correlated with kinase activity.

For detailed protocols on strip preparation, single-dose inhibition profiles, and creating dose-response curves, see *The Kinase Selectivity Profiling System* Technical Manual #TM421, available at www.promega.com/protocols/tm421



Preparation of Kinase and ATP/Substrate Working Stocks:

- Add 95µl of 2.5X Kinase Buffer to all tubes in the Kinase Strip.
- Add 15µl of 100µM ATP to all tubes in the Substrate/Cofactors Strip.

Single-Dose Inhibition Profile:

- Setup Kinase Reactions and No Compound Controls:
 - 1µl of compound or vehicle (5% DMSO)
 - 2µl of Kinase Working Stock
- Setup No Kinase Controls:
 - 1µl vehicle (5% DMSO)
 - 2µl of Kinase Buffer
- Incubate at room temperature for 10 minutes.
- Add 2µl of ATP/Substrate Working Stock.
- Incubate at room temperature for 60 minutes.
- Perform ADP detection using ADP-Glo™ Kinase Assay.

Dose-Response Curves:

- Setup Kinase Reactions:
 - 1µl of 5X compound serial dilution
 - 2µl of Kinase Working Stock
- Setup No Kinase Controls:
 - 2µl of Kinase Buffer in place of Kinase Working Stock
- Incubate at room temperature for 10 minutes.
- Add 2µl of ATP/Substrate Working Stock.
- Incubate at room temperature for 60 minutes.
- Perform ADP detection using ADP-Glo™ Kinase Assay.

A

		Enzastaurin	GSK690693
KSPS: AGC-2	PKCα	5	49
	PKCβ II	4	24
	PKCδ	11	40
	PKCε	9	21
	PKCγ	14	39
	PKCι	97	85
	PKCθ	-3	8
	PKCζ	92	75

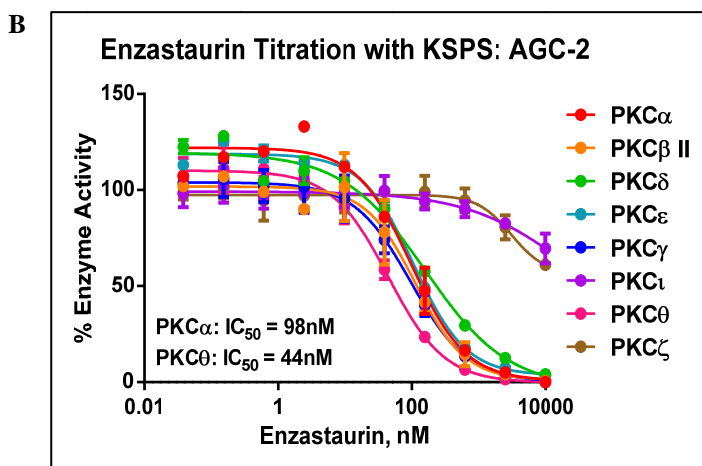


Figure 2. KSPS: AGC-2 Profiling Data. (A) KSPS: AGC-2 kinase activities were determined in the presence of 1µM Enzastaurin or GSK690693. % Activity values were calculated using No Compound and No Kinase Controls and are shown above. Red < 20%; White 20-60%; Blue > 60%. (B) Enzastaurin dose response curves were created with KSPS: AGC-2 to determine the potency (IC₅₀) and selectivity of the inhibitor. IC₅₀ values are comparable to literature values⁽¹⁾.

⁽¹⁾ Graff, J. R. et. al.; Canc. Res., 2005, 65, 7462

Assay Components and Ordering Information:



Products

ADP-Glo™ Kinase Assay
 Kinase Selectivity Profiling System: AGC-2
 Kinase Selectivity Profiling System: AGC-2 + ADP-Glo™ Assay

Company

Promega V6930
 Promega V6910
 Promega V6931

Cat.#

Kinase Buffer: 40mM Tris, pH 7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50µM DTT.