



## ADP-Glo™ Kinase Profiling Application Notes

# AGC KINASE KSPS: AGC-1

# Kinase Selectivity Profiling System: AGC-1

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

## Scientific Background:

Kinase Selectivity Profiling System AGC-1 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).

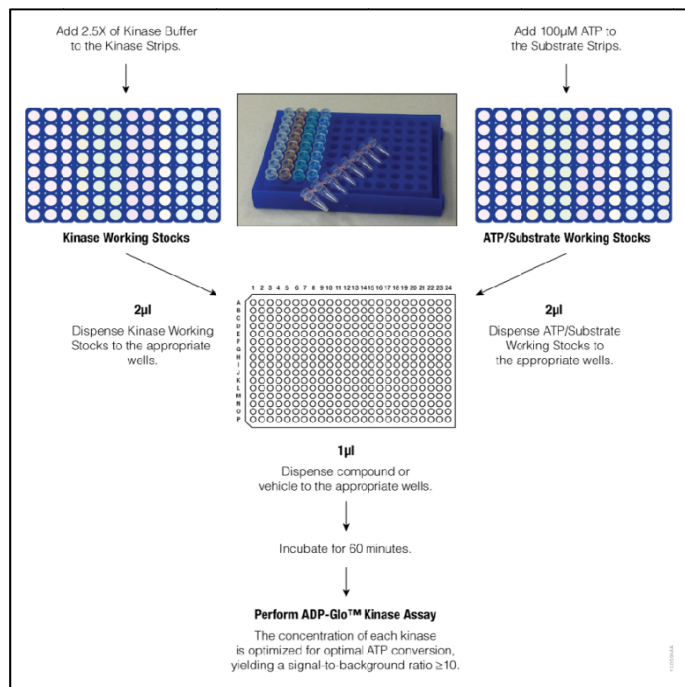
AGC-1		
AGC Family		
	Kinase Strip	Substrate Strip
A	AKT1	AKT (PKB) Substrate
B	p70S6Kβ	RSK Substrate
C	PDK1	PDKtide
D	PKA	Kemptide
E	PKC	Neurogranin + Lipid Activator
F	PRKG1	RSK Substrate + cGMP
G	ROCK1	S6K Substrate
H	RSK2	RSK Substrate

## 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](http://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:

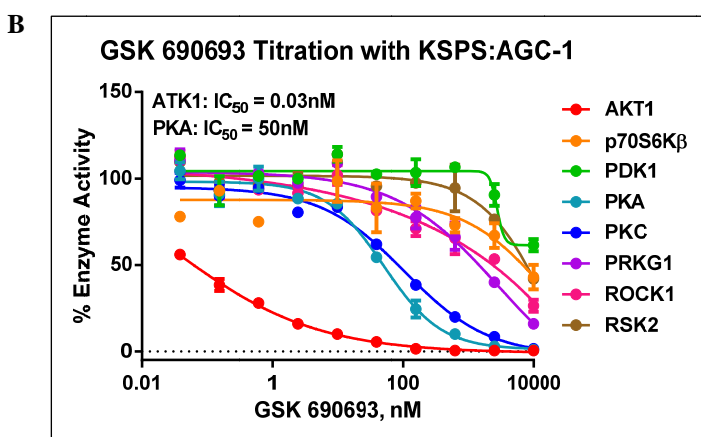
- Setup Kinase Reactions and No Compound Controls:
  - 1µl of compound or vehicle (5% DMSO)
  - 2µl of Kinase Working Stock
- Setup No Kinase Controls:
  - 1µl vehicle (5% DMSO)
  - 2µl of Kinase Buffer
- Incubate at room temperature for 10 minutes.
- Add 2µl of ATP/Substrate Working Stock.
- Incubate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

		GSK690693	Enzastaurin
KSPS: AGC-1	AKT1	2	82
	p70S6Kβ	57	46
	PDK1	70	72
	PKA	9	96
	PKC	22	28
	PRKG1	55	97
	ROCK1	54	87
	RSK2	88	63



**Figure 2. KSPS: AGC-1 Profiling Data.** (A) KSPS: AGC-1 kinase activities were determined in the presence of 1µM GSK690693 or Enzastaurin. % Activity values were calculated using No Compound and No Kinase Controls and are shown above. Red < 20%; White 20-60%; Blue > 60%. (B) GSK690693 dose response curves were created with KSPS: AGC-1 to determine the potency (IC<sub>50</sub>) and selectivity of the inhibitor. IC<sub>50</sub> values are comparable to literature values<sup>(1)</sup>.

<sup>(1)</sup> Rhodes, N. et. al.; Cancer Res, 2008, 68, 2366.

### Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V6930
Kinase Selectivity Profiling System: AGC-1	Promega	V6858
Kinase Selectivity Profiling System: AGC-1 + ADP-Glo™ Assay	Promega	V6859

Kinase Buffer: 40mM Tris, pH 7.5; 20mM MgCl<sub>2</sub>; 0.1mg/ml BSA; 50µM DTT.