



ADP-Glo™ Kinase Profiling Application Notes

Other/CK KINASE KSPS: Other/CK-1

Kinase Selectivity Profiling System: Other/CK-1

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Scientific Background:

Kinase Selectivity Profiling System Other/CK-1 is a set of kinases from the Other/CK 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).

Other/CK-1		
Other/CK Family		
	Kinase Strip	Substrate Strip
A	Aurora A	MBP
B	Aurora B	MBP
C	CK2α1	Casein
D	DNA-PK	DNA-PK Peptide + DNA Activator
E	CK1α1	De-Phospho Casein
F	CK1ε	De-Phospho Casein
G	CK1γ1	Casein
H	VRK2	Casein

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).

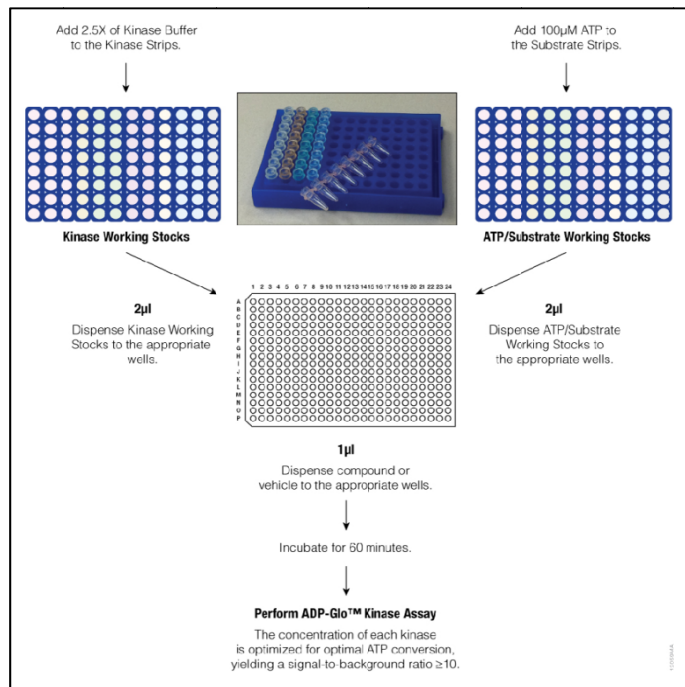


Figure 1. Kinase Selectivity Profiling System Overview. Kinases are provided at either 25X or 50X concentrations in an 8-tube strip, and substrates/cofactors are provided at 3.3X concentrations in a separate 8-tube strip. One-step dilutions directly into the strips produce sufficient Kinase and ATP/Substrate 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

		Tozasertib	IKK 16
KSPS: Other/CK-1	Aurora A	4	20
	Aurora B	2	22
	CK2α1	99	80
	DNA-PK	115	105
	CK1α1	107	103
	CK1ε	107	90
	CK1γ1	104	101
	VRK2	109	103

B

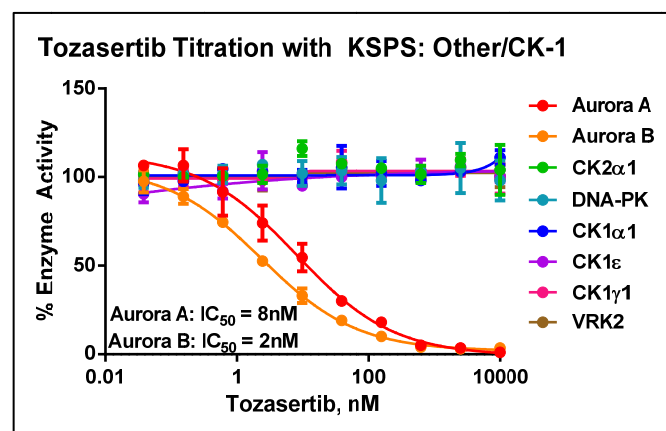


Figure 2. KSPS: Other/CK-1 Profiling Data. (A) KSPS: Other/CK-1 kinase activities were determined in the presence of 1µM Tozasertib or IKK 16. % Activity values were calculated using No Compound and No Kinase Controls and are shown above. Red < 20%; White 20-60%; Blue > 60%. (B) Tozasertib dose response curves were created with KSPS: Other/CK-1 to determine the potency (IC₅₀) and selectivity of the inhibitor. IC₅₀ values are comparable to literature values⁽¹⁾.

⁽¹⁾ Cheetman, G.M.T., et. al.; Cancer Letters. 2007, 251, 323

Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V6930
Kinase Selectivity Profiling System: Other/CK-1	Promega	V6918
Kinase Selectivity Profiling System: Other/CK-1 + ADP-Glo™ Assay	Promega	V6919

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