ADP-Glo™ Kinase Profiling Application Notes

CMGC KINASE KSPS: CMGC-1

Kinase Selectivity Profiling System: CMGC-1

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Scientific Background:

Kinase Selectivity Profiling System CMGC-1 is a set of kinases from the CMGC 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).

CMGC-1		
CMGC Family		
	Kinase Strip	Substrate Strip
A	ERK2	MBP
B	GSK3β	GSK3 Substrate
C	JNK1	p38 Substrate
D	JNK3	p38 Substrate
E	p38α	p38 Substrate
F	p38β	p38 Substrate
G	p38δ	p38 Substrate
H	p38γ	p38 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).

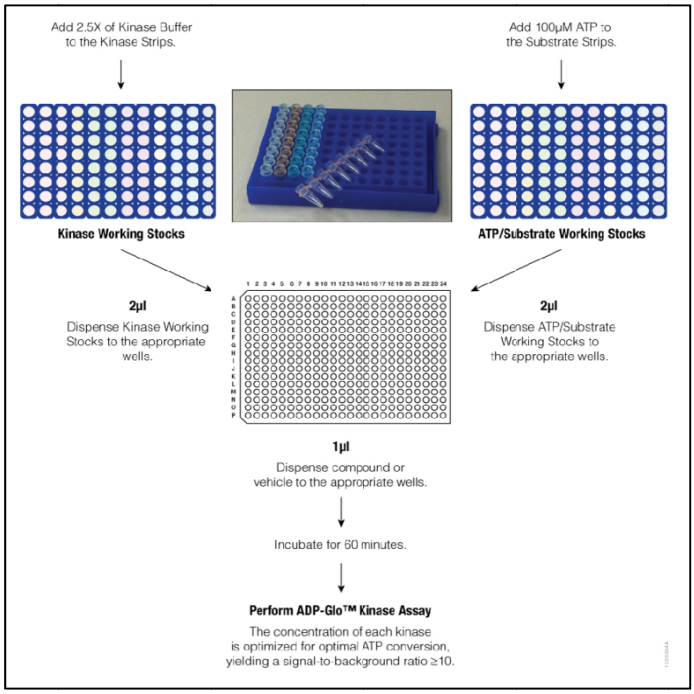

Figure 1. Kinase Selectivity Profiling System Overview. Kinases are provided at either 25X or 50X concentrations in an 8-tube strip, and substrates/cofactors are provided at 3.3X concentrations in a separate 8-tube strip. One-step dilutions directly into the strips produce sufficient Kinase and ATP/Substrate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

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

		SB203580	Roscovitine
KSPS: CMGC-1	ERK2	97	99
	GSK3β	55	96
	JNK1	84	107
	JNK3	30	106
	p38α	3	101
	p38β	10	101
	p38δ	105	107
	p38γ	94	104

B

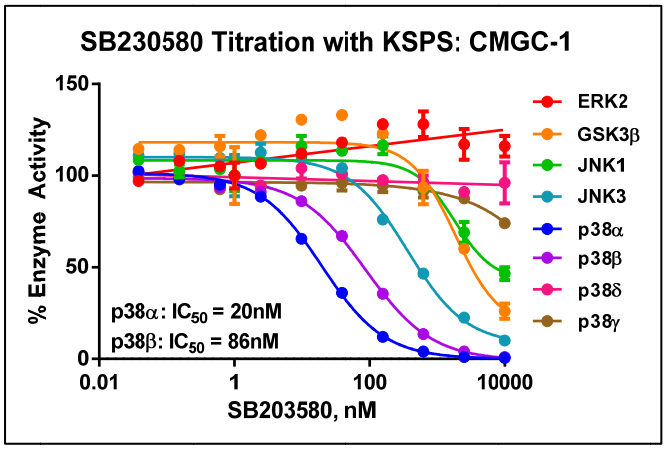

Figure 2. KSPS: CMGC-1 Profiling Data. (A) KSPS: CMGC-1 kinase activities were determined in the presence of 1µM SB203580 or Roscovitine. % Activity values were calculated using No Compound and No Kinase Controls and are shown above. Red < 20%; White 20-60%; Blue > 60%. (B) SB203580 dose response curves were created with KSPS: CMGC-1 to determine the potency (IC50) and selectivity of the inhibitor. IC50 values are comparable to literature values [1].

[1] Cuenda, A. et. al.; FEBS Letters. 1995, 364, 229.

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V6930
Kinase Selectivity Profiling System: CMGC-1	Promega	V6854
Kinase Selectivity Profiling System: CMGC-1 + ADP-Glo™ Assay	Promega	V6855

Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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