



## ADP-Glo™ Kinase Profiling Application Notes

# CAMK KINASE KSPS: CAMK-1

# Kinase Selectivity Profiling System: CAMK-1

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## Scientific Background:

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

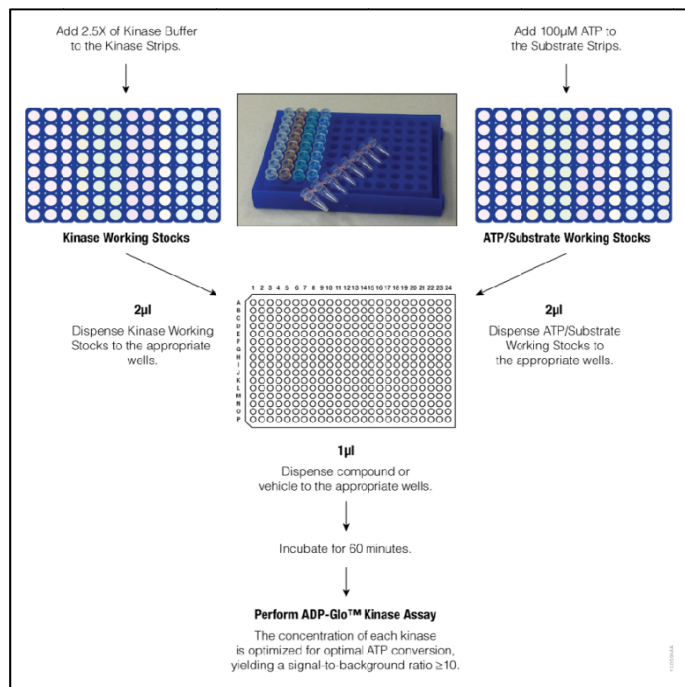
CAMK-1		
CAMK Family		
	Kinase Strip	Substrate Strip
A	CHK1	CHKtide
B	CHK2	CHKtide
C	MAPKAPK2	HSP27tide
D	MARK1	CHKtide
E	MELK	ZIPtide
F	PASK	ZIPtide
G	PIM1	S6K Substrate
H	PKC $\mu$ (PKD1)	CREBtide

## 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](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.

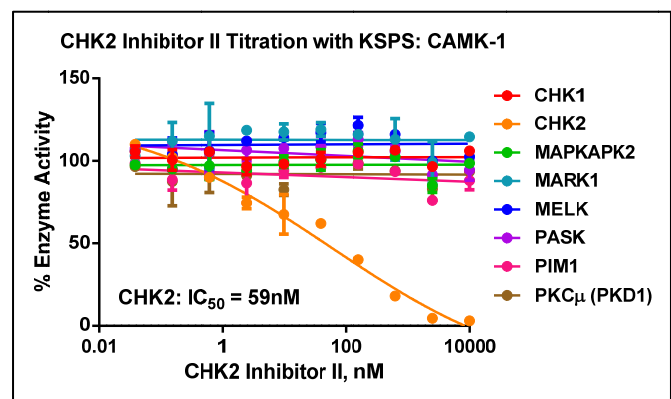
#### Dose-Response Curves:

- 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

		CHK2 Inhibitor II	Dorsomorphin
KSPS: CAMK-1	CHK1	100	68
	CHK2	15	60
	MAPKAPK2	100	95
	MARK1	111	73
	MELK	98	8
	PASK	100	88
	PIM1	82	74
	PKCµ (PKD1)	95	72

B



**Figure 2. KSPS: CAMK-1 Profiling Data.** (A) KSPS: CAMK-1 kinase activities were determined in the presence of 1µM CHK2 Inhibitor II or Dorsomorphin. % Activity values were calculated using No Compound and No Kinase Controls and are shown above. Red < 20%; White 20-60%; Blue > 60%. (B) CHK2 Inhibitor II dose response curves were created with KSPS: CAMK-1 to determine the potency (IC<sub>50</sub>) and selectivity of the inhibitor. IC<sub>50</sub> values are comparable to literature values (1).

(1) Arienti, K. L. et al; J. Med. Chem., 2005, 48, 1873.

#### Assay Components and Ordering Information:



#### Products

ADP-Glo™ Kinase Assay  
 Kinase Selectivity Profiling System: CAMK-1  
 Kinase Selectivity Profiling System: CAMK-1 + ADP-Glo™ Assay

#### Company

Promega  
 Promega  
 Promega

#### Cat.#

V6930  
 V6932  
 V6913

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