ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: SIK

SIK Kinase Assay

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Scientific Background:

SIK is a protein kinase that is involved in regulating AMPK-related kinases (1). SIK may mediate the physiological effects of LKB1, including its tumour suppressor function. SIK is also involved in signaling by various proteins like STRAD, NUAK1, NUAK2, BRSK1, BRSK2, QIK, QSK, SIK, MARK1, MARK2, MARK3, MARK4 and MELK that are related to AMPK. Activation of SIK1 by phosphorylation on thr322 can lead to an increase in the catalytic activity of sodium/potassium ATPase alpha subunit at the plasma membrane (2). This results in an increase in intracellular sodium in intact mammalian cells.

1. Takemori, H. Et al: TORC-SIK cascade regulates CREB activity through the basic leucine zipper domain. FEBS J. 2007; 274 (13): 3202–9.

2. Sjostrom, M.; SIK1 is part of a cell sodium-sensing network that regulates active sodium transport through a calcium-dependent process. Proc. Nat. Acad. Sci. 104: 16922-16927, 2007.

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 (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). 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. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

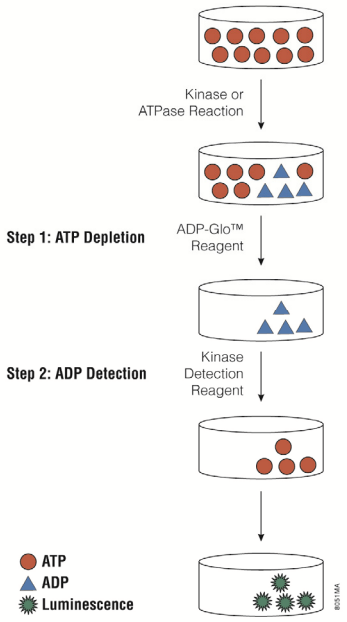


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

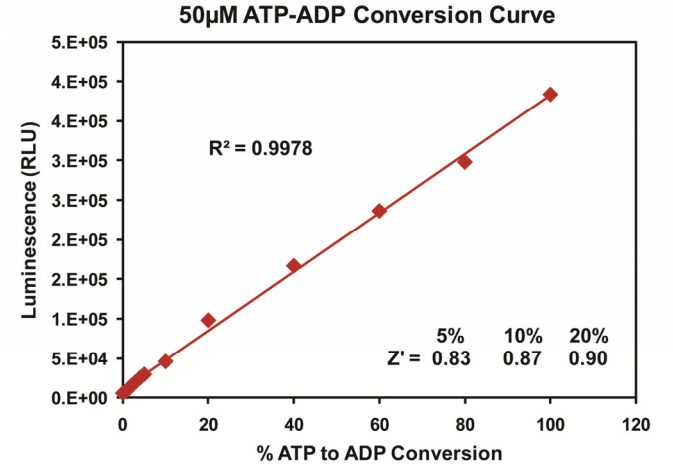


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

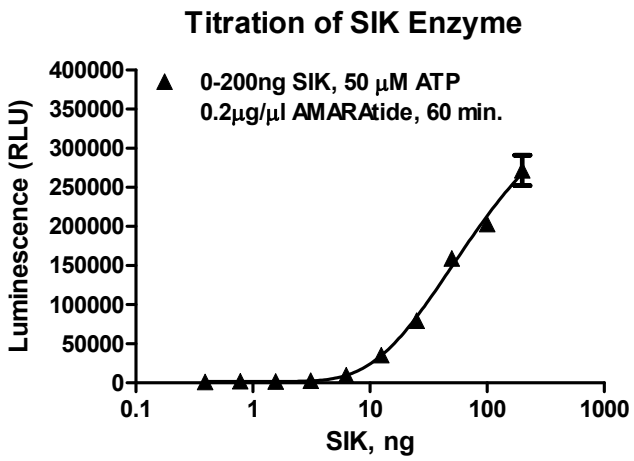
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1 µl of inhibitor or (5% DMSO)
  - 2 µl of enzyme (defined from table 1)
  - 2 µl of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 µl of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 µl of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. SIK Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

SIK, ng	200	100	50	25	13	6.3	3.1	1.6	0
RLU	271787	203199	159162	79356	35369	9965	2881	1798	950
S/B	286	214	168	84	37	10	3	2	1
% Conversion	65	49	38	19	8	2.16	0.45	0.19	0



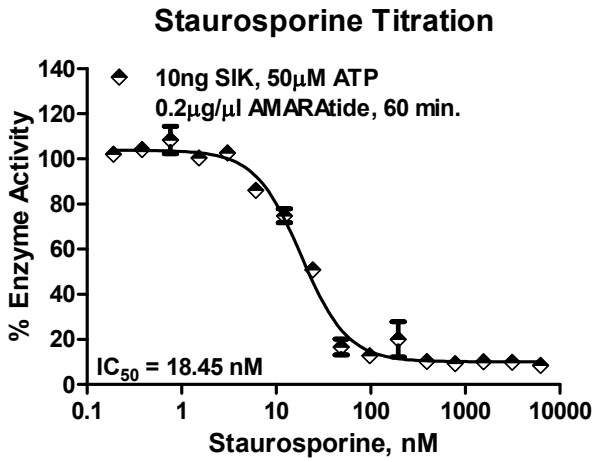


Figure 3. SIK Kinase Assay Development. (A) SIK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of SIK to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
SIK Kinase Enzyme System	Promega	V4156
ADP-Glo™ + SIK Kinase Enzyme System	Promega	V4157

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