ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: SLK

SLK Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

SLK or LOSK is a Ste20-like protein kinase that associates with microtubule and centrosome. Inhibition of SLK activity by dominant-negative mutant or RNAi leads to unfocused microtubule arrangement indicating that SLK is needed for microtubule organization and for the proper localization of Golgi complex (1). Expression of v-Src can down-regulate SLK activity through the involvement of CK2 which can directly phosphorylate and inhibit SLK activity whereas inhibition of CK2 in v-Src-transformed cells results in normal SLK activity (2). CK2 and SLK can be co-localized in fibroblasts spreading on fibronectin-coated substrates.

1. Burakov A V, et al: LOSK (SLK) protein kinase activity is necessary for microtubule organization in the interphase cell centrosome. Dokl Biol Sci. 2005;403:317-9

2. Chaar Z, et al: v-Src-dependent down-regulation of the Ste20-like kinase SLK by casein kinase II. J Biol Chem. 2006 Sep 22;281(38):28193-9.

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.

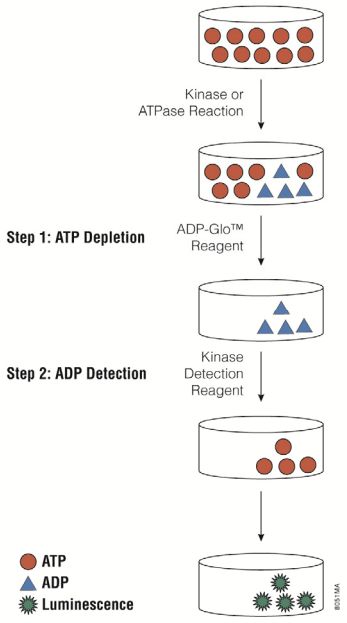

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

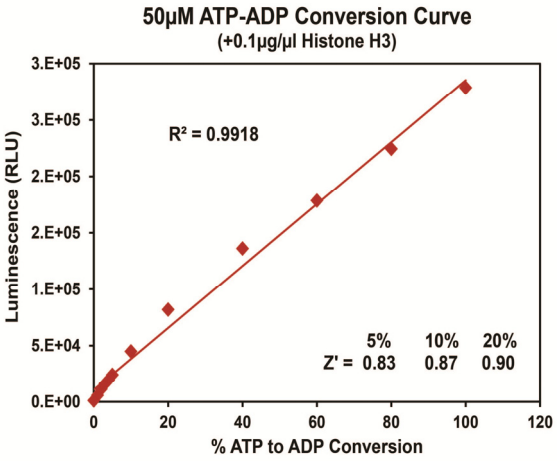

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. SLK 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.

SLK, ng	100	50	25	12.5	6.25	3.13	1.56	0.78	0
RLU	44996	38730	26664	16015	8476	5458	3075	2183	1180
S/B	38	33	23	14	7	5	3	2	1
% Conversion	9	8	5	3	2	0.9	0.4	0.2	0

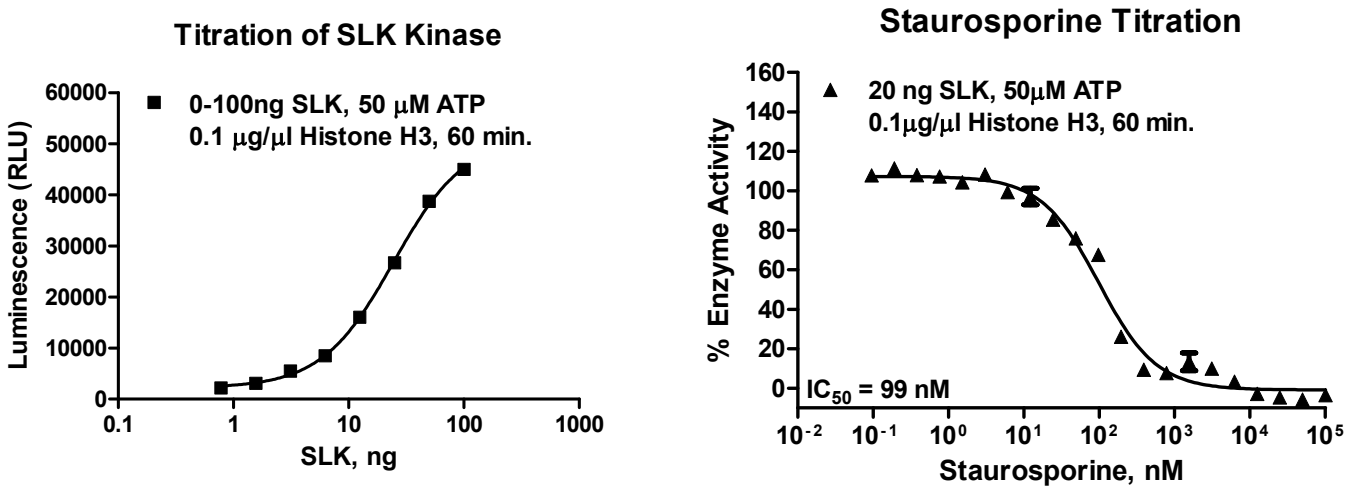

Figure 3. SLK Kinase Assay Development. (A) SLK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 20ng of SLK to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
SLK Kinase Enzyme System	Promega	V4242
ADP-Glo™ + SLK Kinase Enzyme System	Promega	V4243

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