SGK1 Kinase Assay

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Scientific Background:

SGK1 is a member of the serum- and glucocorticoid-induced protein kinase family that is activated in vitro by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and in vivo in response to signals that activate phosphatidylinositol (PI) 3-kinase (1). SGK1 mRNA is expressed in all tissues and the level of SGK1 mRNA is increased by stimulation with serum or dexamethasone. SGK1 promotes cell survival by phosphorylating and inactivating FKHRL1 (2). SGK and Akt display differences with respect to the efficacy with which they phosphorylate the three regulatory sites on FKHRL1.

1. Kobayashii, T. et al: Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. Biochem J. 1999 Nov 15;344 Pt 1:189-97.
2. Brunet, A. et al: Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). Mol Cell Biol. 2001 Feb;21(3):952-65.

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.

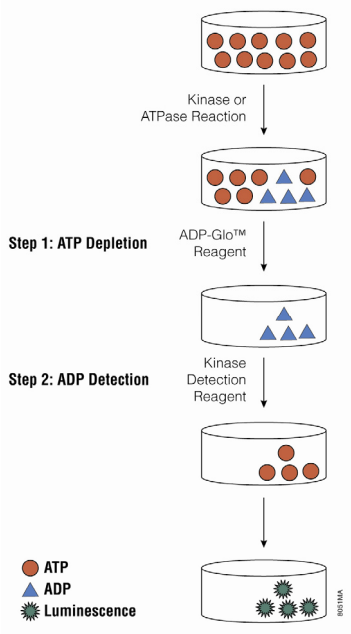

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

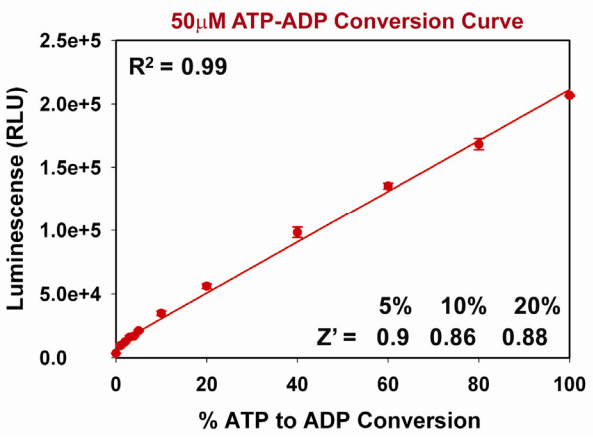

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. SGK1 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.

SGK1, ng	100	50	25	13	6.3	3.1	1.6	0.78	0
RLU	157430	113364	88287	57328	34488	21215	11927	7860	2153
S/B	73	53	41	27	16	10	6	4	1
% Conversion	62	44	34	21	11	6	2	0	0

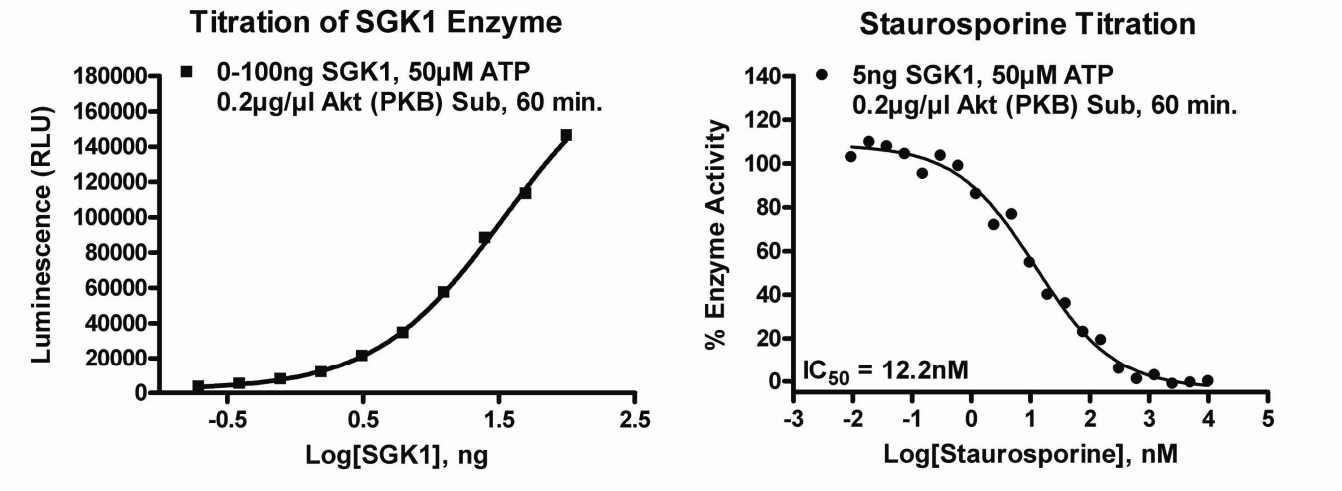

Figure 3. SGK1 Kinase Assay Development. (A) SGK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 5ng of SGK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
SGK1 Kinase Enzyme System	Promega	V2911
ADP-Glo + SGK1 Kinase Enzyme System	Promega	V9671

SGK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.