

SGK2 Kinase Assay

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Scientific Background:

SGK2 is a member of the serum- and glucocorticoid-induced kinases (SGK) which are serine-threonine kinases and belong to the "AGC" kinase subfamily, which includes protein kinases A, G, and C, and its catalytic domain is most similar to protein kinase B (PKB) (1). SGK2, like the other two isoforms SGK1 and SGK3, is stimulated by insulin and insulin-like growth factor-1 (IGF-1), and has been shown to enhance Na(+)/K(+)-ATPase activity in a variety of cells (2).

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. Boehmer, C. et al: Stimulation of renal Na⁺ dicarboxylate cotransporter 1 by Na⁺/H⁺ exchanger regulating factor 2, serum and glucocorticoid inducible kinase isoforms, and protein kinase B. *Biochem Biophys Res Commun.* 2004 Jan 23;313(4):998-1003.

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.

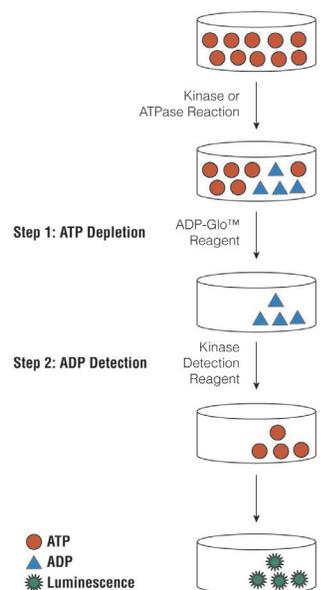


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

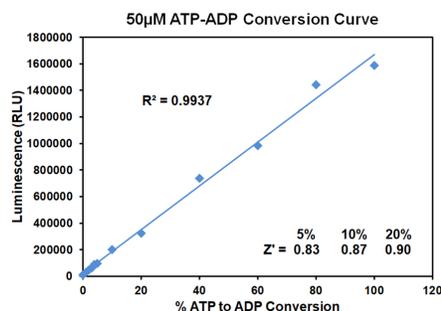


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: <http://www.promega.com/KESProtocol>

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
 - ✓ 1 μ l of inhibitor or (5% DMSO)
 - ✓ 2 μ l of enzyme (defined from table 1)
 - ✓ 2 μ l of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 μ l of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent.
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1 second).

Table 1. 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.

| Enzyme, ng | 300 | 150 | 75 | 37.50 | 18.75 | 9.38 | 4.69 | 2.34 | 1.17 | 0.59 | 0 |
|--------------|-----------|---------|---------|---------|---------|---------|--------|--------|--------|-------|-------|
| Luminescence | 1,093,350 | 908,998 | 716,839 | 460,583 | 276,127 | 137,078 | 62,758 | 27,110 | 12,892 | 6,704 | 3,274 |
| S/B | 334 | 278 | 219 | 141 | 84 | 42 | 19 | 8 | 4 | 2 | 1 |
| % Conversion | 84 | 69 | 54 | 34 | 20 | 9 | 3 | 0 | 0 | 0 | 0 |

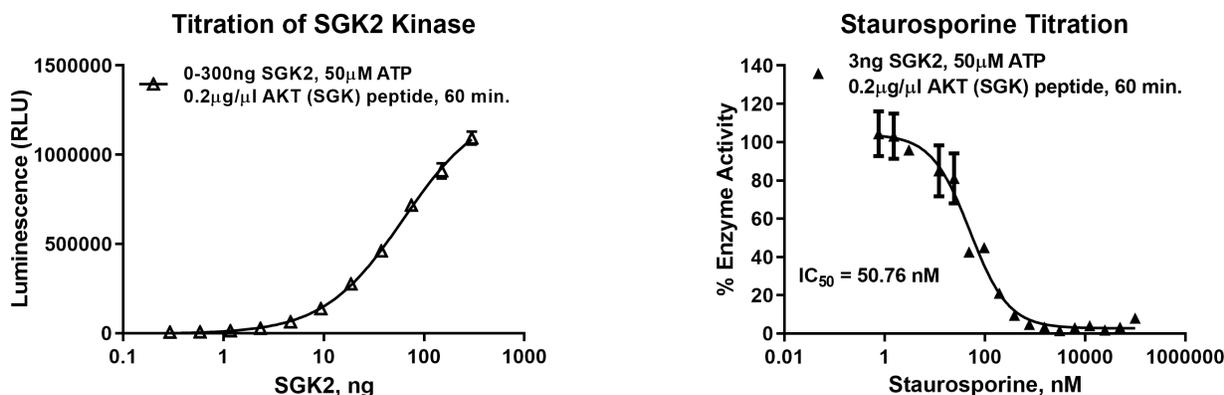


Figure 3. SGK2 Kinase Assay Development. (A) SGK2 enzyme was titrated using 50 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of SGK2 to determine the potency of the inhibitor (IC_{50}).



Ordering Information:

| Products | Size | Cat. # |
|--------------------------------------|------------|--------|
| SGK2 Kinase Enzyme System | 10 μ g | VA7555 |
| | 1mg | VA7556 |
| ADP-Glo™ + SGK2 Kinase Enzyme System | 1 Each | VA7557 |