ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

SBK1 Kinase Assay

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Scientific Background:

SBKI or SH3 domain binding kinase 1 is a novel serine/threonine protein kinase structurally related to a Xenopus gastrula-specific protein kinase, Pk9.7 which possessing a consensus sequence for an SH3-binding domain from developing rat brain and it play an important role in signal-transduction pathways related to the control of brain development (1). SBK1 may predict a broad cellular function, and its dysregulated in certain cancers suggests an involvement of the protein in the pathogenesis of human cancers (2).

1. Wang. P.et.al: Human SBK1 is dysregulated in multiple cancers and promotes survival of ovary cancer SK-OV-3 cells. Mol Biol Rep. 2011 Jun; 38(5):3551-9.

2. Nara.K.et.al: Cloning and characterization of a novel serine/threonine protein kinase gene expressed predominantly in developing brain. Eur J Biochem. 2001 May;268(9):2642-51

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.

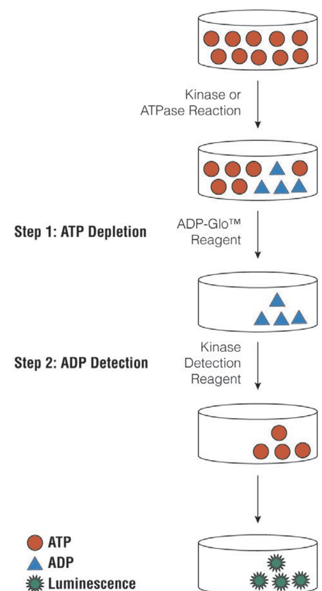

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

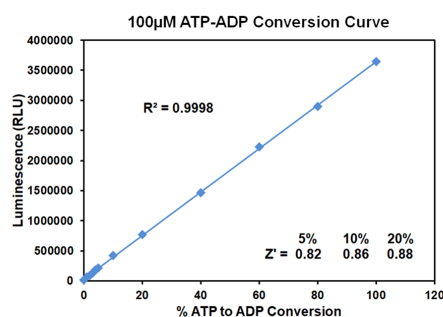

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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 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 indicated time (See Figure 3).
- 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 0.5-1 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	0
Luminescence	3,788,075	2,739,990	1,658,345	713,564	259,275	95,446	45,711	16,292
S/B	233	168	102	44	16	6	3	1
% Conversion	104	75	45	19	6	2	0	0

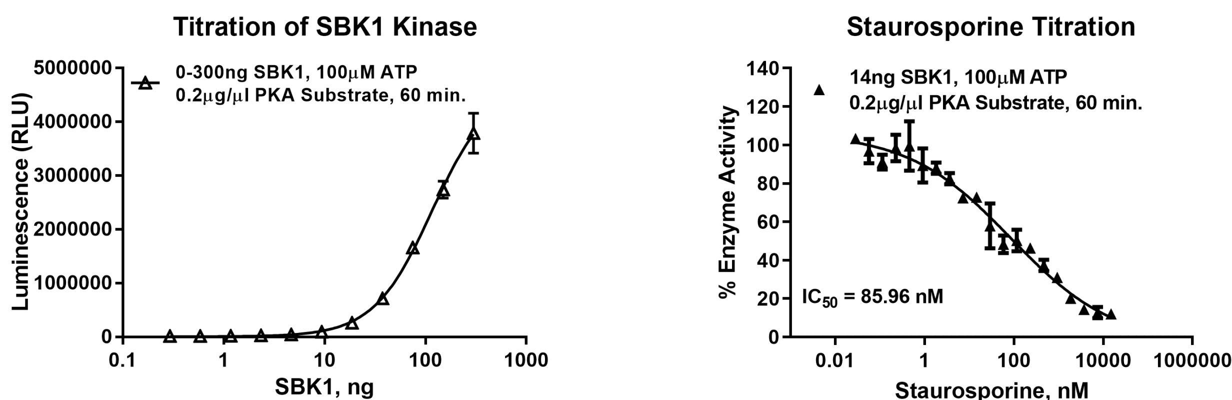

Figure 3. SBK1 Kinase Assay Development. (A) SBK1 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 14ng of SBK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
SBK1 Kinase Enzyme System	10µg	VA7363
	1mg	VA7364
ADP-Glo™ + SBK1 Kinase Enzyme System	1 Each	VA7365