ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

SRPK1 Kinase Assay

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Scientific Background:

SRPK1 is a Ser/Thr protein kinase that has sequence and function similar to SRPK2. In human cancer cells, it was observed that inactivation of SRPK1 induces cellular resistance to anticancer drugs such as cisplatin and bleomycin (1). SRPK1 phosphorylates serine/arginine-rich (SR) proteins, such as splicing factors ASF/SF2, SC35, and SRp55, in their arginine/serine-rich (RS) domains in vitro. Hence it is believed that SRPK1 plays a key role in regulation of both constitutive and alternative splicing by regulating intracellular localization of splicing factors (2).

1. Hayes, G.M. et al: Serine-arginine protein kinase 1 overexpression is associated with tumorigenic imbalance in mitogen-activated protein kinase pathways in breast, colonic, and pancreatic carcinomas. Cancer Res. 2007;67(5):2072-80.

2. Gui, J.-F. et al: A serine kinase regulates intracellular localization of splicing factors in the cell cycle. Nature 369: 678-682, 1994.

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.

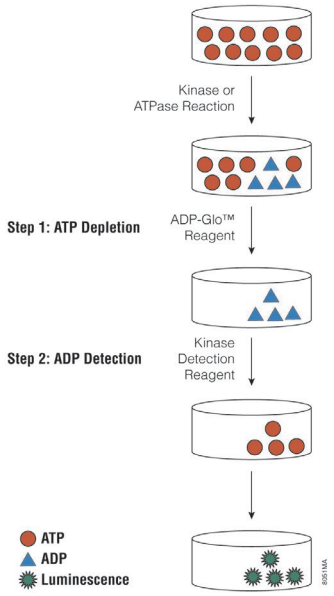

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

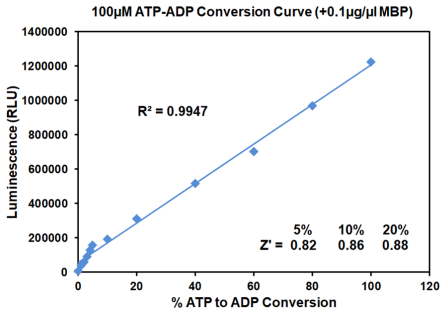

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0
Luminescence	676,566	269,111	168,120	100,329	60,438	34,016	18,676	11,633	7,568	3,900
S/B	173	69	43	26	16	9	5	3	2	1
% Conversion	54	19	10	4	0	0	0	0	0	0

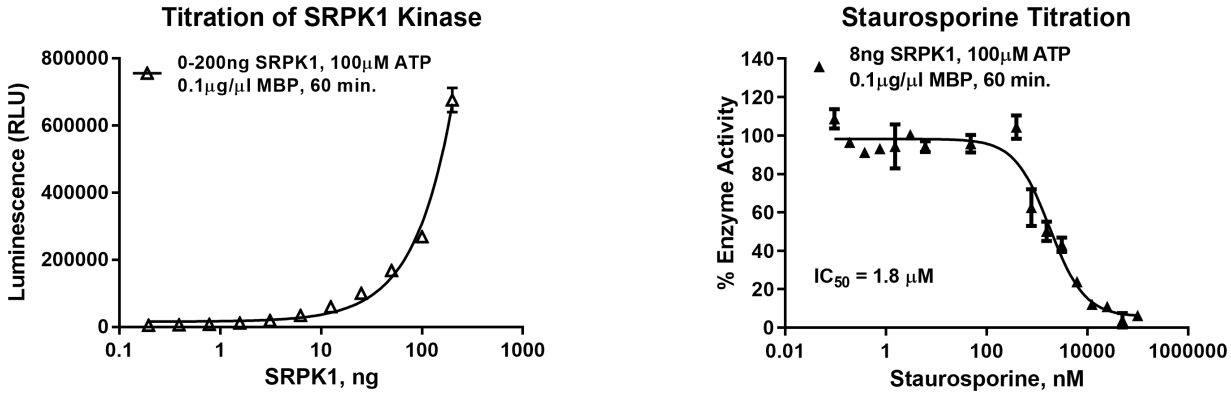

Figure 3. SRPK1 Kinase Assay Development. (A) SRPK1 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 8ng of SRPK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
SRPK1 Kinase Enzyme System	10µg	VA7558
	1mg	VA7559
ADP-Glo™ + SRPK1 Kinase Enzyme System	1 Each	VA7560