ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

SRPK2 Kinase Assay

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Scientific Background:

SRPK2 is a member of the serine/arginine (SR) protein-specific kinase family that are cell cycle-regulated protein kinases which phosphorylate SR domain-containing proteins in nuclear speckles and mediate the pre-mRNA splicing events (1). SRPK2 knock down results in hypophosphorylation of the serine/arginine domain-containing human PRP28 protein thereby destabilizing PRP28 association with the tri-snRNP. RNAi-mediated depletion in HeLa cells showed that SRPK2 is essential for cell viability, and it is required for spliceosomal B complex formation (2).

1. Wang, H. Y. Et al; SRPK2: a differentially expressed SR protein-specific kinase involved in mediating the interaction and localization of pre-mRNA splicing factors in mammalian cells. J. Cell Biol. 140: 737-750, 1998.

2. Mathew, R. et al: Phosphorylation of human PRP28 by SRPK2 is required for integration of the U4/U6-U5 tri-snRNP into the spliceosome.Nat Struct Mol Biol. 2008 May;15(5):435-43.

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.

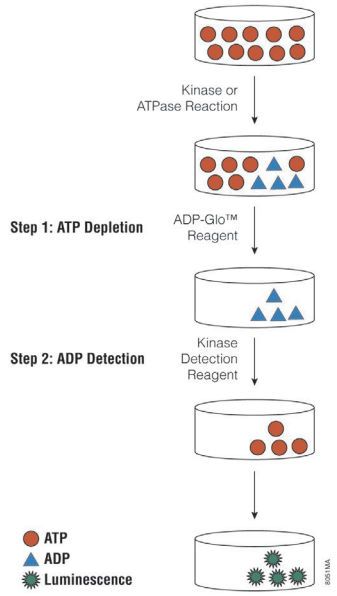

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

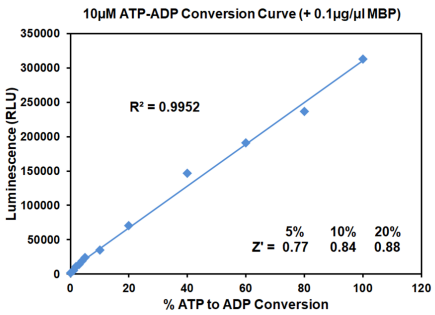

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	1.88	0.94	0.47	0
Luminescence	191,448	121,108	90,945	48,109	25,049	14,450	8,612	4,413	2,827	1,636	850
S/B	225	142	107	57	29	17	10	5	3	2	1
% Conversion	65	40	30	15	7	3	1	0	0	0	0

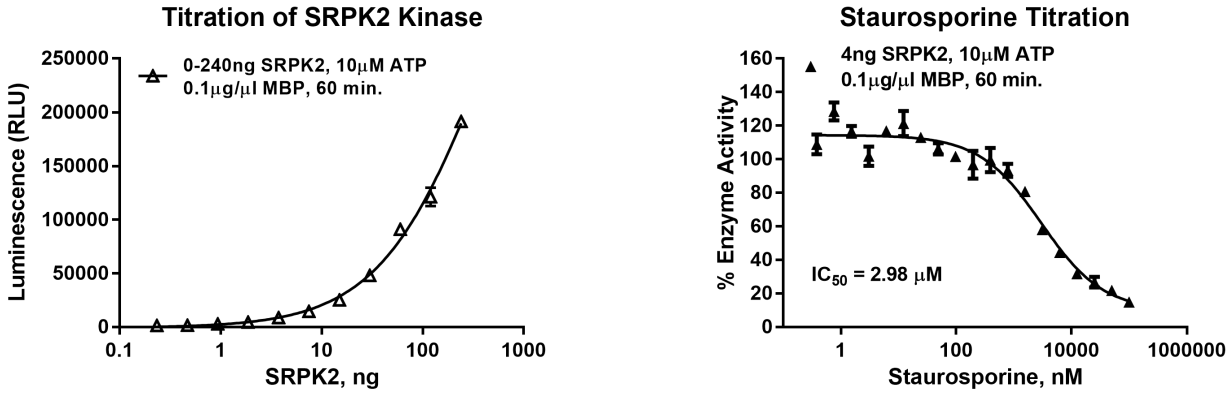

Figure 3. SRPK2 Kinase Assay Development. (A) SRPK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 4ng of SRPK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
SRPK2 Kinase Enzyme System	10µg	VA7561
	1mg	VA7562
ADP-Glo™ + SRPK2 Kinase Enzyme System	1 Each	VA7563