ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

MSSK1 Kinase Assay

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Scientific Background:

MSSK1, also known as SRPK3, is a muscle-specific protein kinase belonging to the serine arginine protein kinase family which phosphorylates serine/arginine repeat-containing proteins. Heart and skeletal muscle show high expression of the MSSK1/SRPK3 gene product. SRPK3-null mice display a new entity of type 2 fiber-specific myopathy with a marked increase in centrally placed nuclei. Transgenic mice overexpressing SRPK3 in skeletal muscle show severe myofiber degeneration and early lethality (1).

1. Nakagawa, O. et al: Centronuclear myopathy in mice lacking a novel muscle-specific protein kinase transcriptionally regulated by MEF2. Genes Dev. 2005 Sep 1;19(17):2066-77.

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.

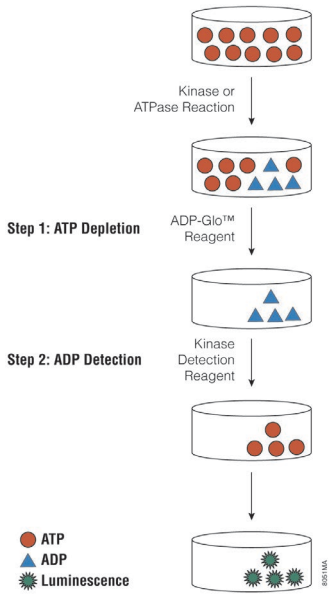

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

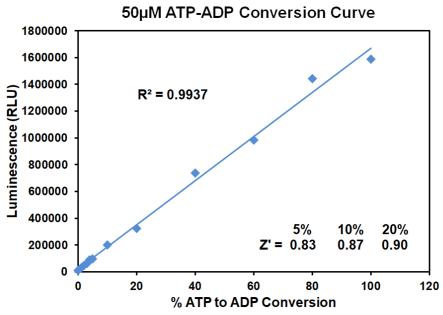

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	1.88	0.94	0
Luminescence	905,011	468,333	290,250	134,143	64,627	44,928	11,300	10,605	5,999
S/B	151	78	48	22	11	7	1.88	2	1
% Conversion	69	35	21	9	3	2	0	0	0

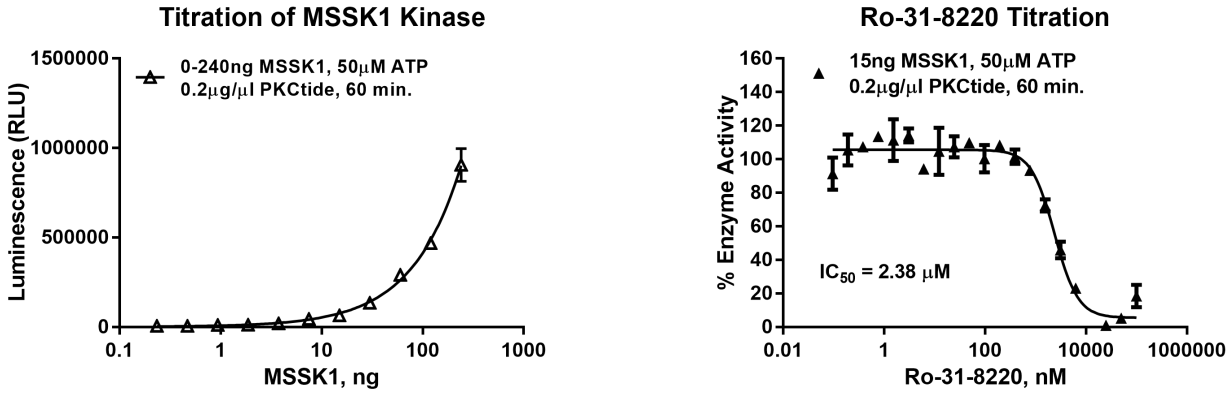

Figure 3. MSSK1 Kinase Assay Development. (A) MSSK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 15ng of MSSK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MSSK1 Kinase Enzyme System	10µg	VA7507
	1mg	VA7508
ADP-Glo™ + MSSK1 Kinase Enzyme System	1 Each	VA7509