ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

NIM1 Kinase Assay

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Scientific Background:

NIM1 belongs to CAMK Ser/Thr protein kinase family. Unlike to other AMPK-related kinases, NIM1 cannot be phosphorylated and activated by LKB1 (1). Two somatic mutations (P333S and P411T) were found in lung neuroendocrine carcinoma and lung large cell carcinoma samples (2).

1. Jaleel M. et.al: Identification of the sucrose non-fermenting related kinase SNRK, as a novel LKB1 substrate. FEBS Lett. 579:1417-1423(2005).

2. Greenman C. et.al: Patterns of somatic mutation in human cancer genomes. Nature 446:153-158(2007).

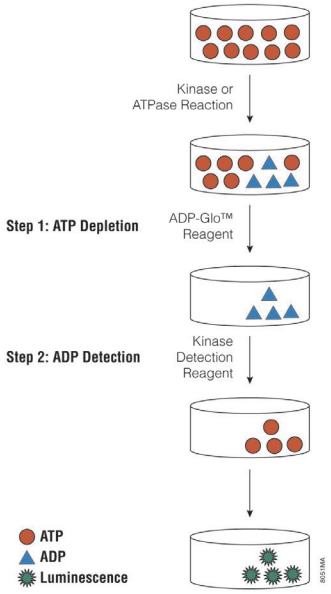

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

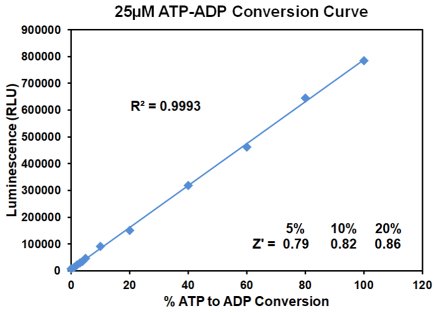

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	180	90	45	22.50	11.25	5.63	2.81	1.41	0.70	0.35	0
Luminescence	1,052,140	902,715	733,044	567,734	335,467	153,168	64,808	31,220	18,560	10,631	7,050
S/B	149	128	104	81	48	22	9	4	3	2	1
% Conversion	116	100	81	62	36	16	6	3	1	0	0

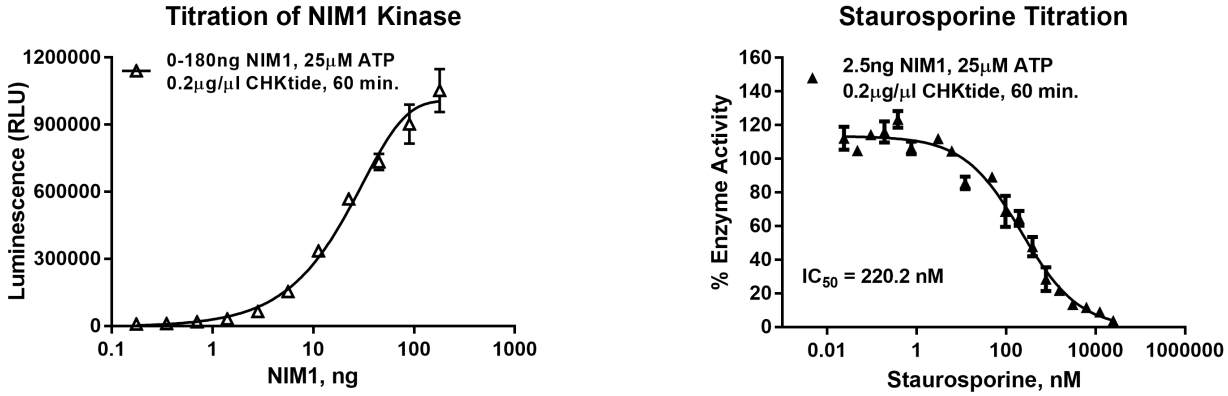

Figure 3. NIM1 Kinase Assay Development. (A) NIM1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2.5ng of NIM1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
NIM1 Kinase Enzyme System	10µg	VA7249
	1mg	VA7250
ADP-Glo™ + NIM1 Kinase Enzyme System	1 Each	VA7251