ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

NEK9 Kinase Assay

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Scientific Background:

NEK9 is a member of the NEK family and has high homology to NEK1, NEK3 and NEK4. NEK9 is activated during mitosis and binds specifically to RAN GTPase and is a substrate for CDC2 phosphorylation (1). NEK9 plays a role in the control of mitotic progression and is regulated by CDC2 and RAN GTPase. Overexpression of both active and inactive variants of NEK9 is toxic to cells and inhibits cell division causing abnormal nuclear morphologies. NEK9 can catalyze the phosphorylation of recombinant NEK6 and NEK7 in vitro leading to its activation. This suggests that NEK9 may be responsible for activation of NEK6 and NEK7 during mitosis (2).

1. Roig J, et al: Nercc1, a mammalian NIMA-family kinase, binds the Ran GTPase and regulates mitotic progression. Genes Dev. 16: 1640-1658, 2002.

2. Belham C, et al: A mitotic cascade of NIMA family kinases: Nercc1/Nek9 activates the Nek6 and Nek7 kinases. J. Biol. Chem. 278: 34897-34909, 2003.

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.

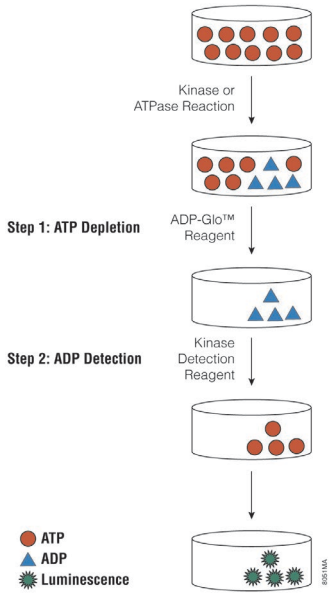

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

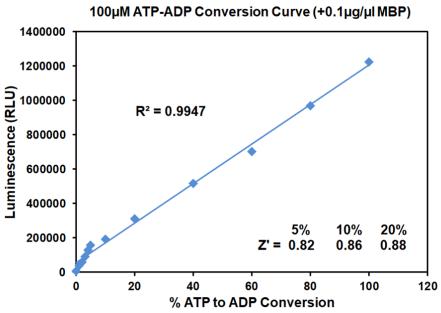

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	0
Luminescence	586,279	254,241	127,181	62,170	31,315	14,640	9,429	5,862
S/B	100	43	22	11	5	2	2	1
% Conversion	60	23	8	1	0	0	0	0

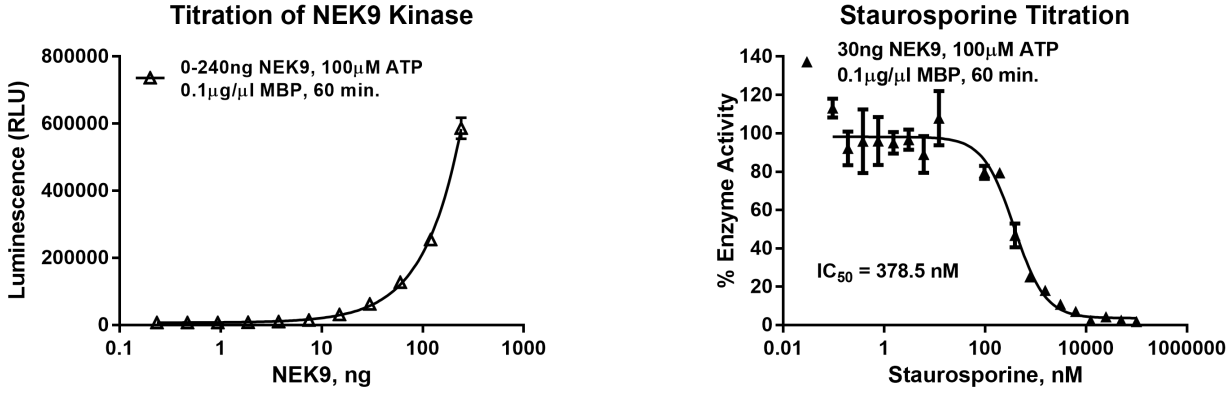

Figure 3. NEK9 Kinase Assay Development. (A) NEK9 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 30ng of NEK9 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
NEK9 Kinase Enzyme System	10µg	VA7516
	1mg	VA7517
ADP-Glo™ + NEK9 Kinase Enzyme System	1 Each	VA7518