ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

NEK7 Kinase Assay

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Scientific Background:

NEK7 is a member of the NIMA family of serine/threonine kinases. In contrast to the other documented NIMA-related kinases, NEK7 harbor its catalytic domain in the C-terminus of the protein. Immunofluorescence studies suggest that NEK7 is cytoplasmic and located on chromosome 1 (1). The major protein kinase that is active on the p70 S6 kinase hydrophobic regulatory site (FXXFS/TF/Y) Thr412, was purified from rat liver and identified as NEK7 (2). NEK7 kinase activity is rapidly and efficiently increased by serum deprivation, and may be regulated in a cell cycle-dependent manner.

1. Kandli, M. et al: Isolation and characterization of two evolutionarily conserved murine kinases (Nek6 and nek7) related to the fungal mitotic regulator, NIMA. Genomics. 2000 Sep 1;68(2):187-96.

2. Belham, C. et al: Identification of the NIMA family kinases NEK6/7 as regulators of the p70 ribosomal S6 kinase. Curr Biol. 2001 Aug 7;11(15):1155-67

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.

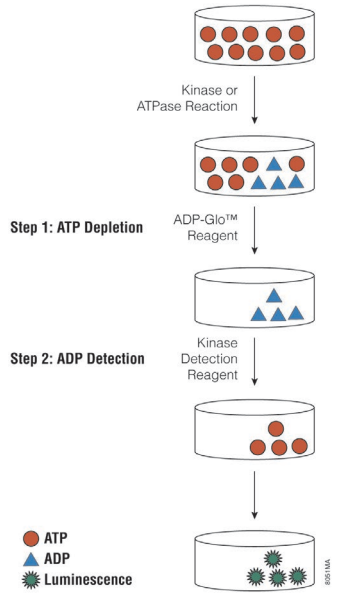

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

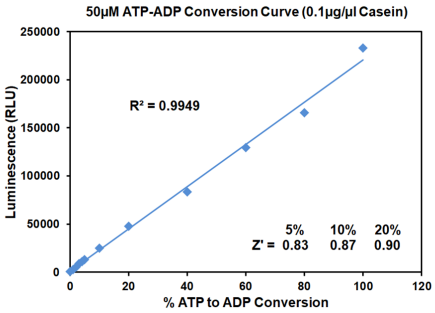

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0
Luminescence	147,691	55,770	32,263	15,299	8,722	5,281	2,330	1,411	531
S/B	278	105	61	29	16	10	4	3	1
% Conversion	67	25	14	6	4	2	1	0	0

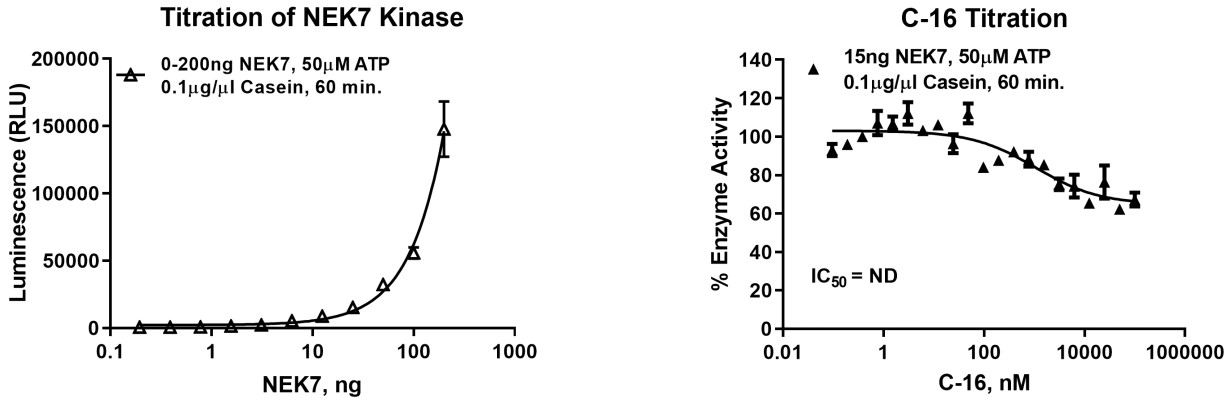

Figure 3. NEK7 Kinase Assay Development. (A) NEK7 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 15ng of NEK7 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
NEK7 Kinase Enzyme System	10µg	VA7513
	1mg	VA7514
ADP-Glo™ + NEK7 Kinase Enzyme System	1 Each	VA7515