ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

NEK1 Kinase Assay

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Scientific Background:

NEK1 or NIMA (never in mitosis gene a)-related kinase 1 is a serine/threonine kinase involved in cell cycle regulation and is found in a centrosomal complex with FEZ1, a neuronal protein that plays a role in axonal development. NEK1 is involved early in the DNA damage response pathway (1). NEK1 cycles through the nucleus via its nuclear localization and export signals (2). NEK1 protein participates in different signaling pathways to regulate diverse cellular processes and plays an important role in the kidney where it has opened a new avenue for studying cystogenesis and identifying possible modes of therapy.

1. Polci, R. et.al: NIMA-related protein kinase 1 is involved early in the ionizing radiation-induced DNA damage response. Cancer Res. 64: 8800-8803, 2004.

2. Hilton, L. K. et.al: The NIMA-related kinase NEK1 cycles through the nucleus. Biochem. Biophys. Res. Commun. 389: 52-56, 2009.

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.

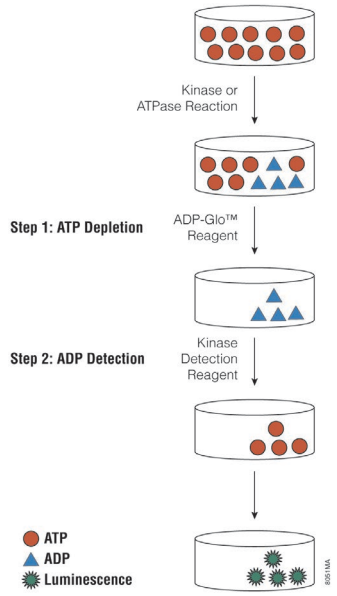

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

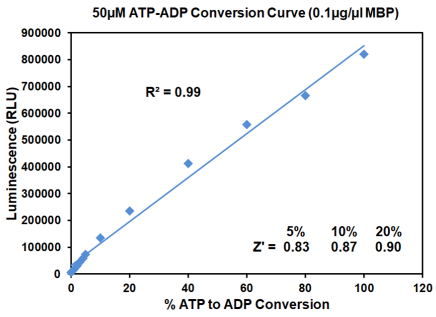

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0
Luminescence	683,452	397,592	167,603	89,910	41,874	20,790	9,899	6,395	3,064
S/B	223	130	55	29	14	7	3	2	1
% Conversion	79	45	16	7	1	0	0	0	0

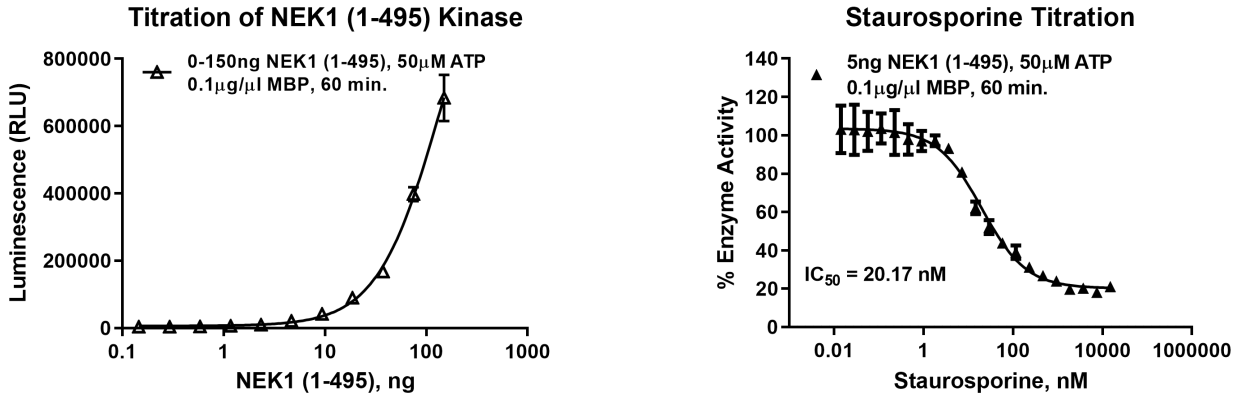

Figure 3. NEK1 (1-495) Kinase Assay Development. (A) NEK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 5ng of NEK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
NEK1 Kinase Enzyme System	10µg	VA7588
	1mg	VA7589
ADP-Glo™ + NEK1 Kinase Enzyme System	1 Each	VA7590