NEK3 Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

NEK3 is a member of the NEK family of protein kinases that share high amino acid homology with NIMA (never in mitosis gene a). NEK3 mRNA is detected in all proliferating cell lines at levels that remain constant during the cell cycle (1). During Prolactin receptor signaling, VAV2 is phosphorylated and activated by NEK3. Overexpression of NEK3 in Chinese hamster ovary cells increases cytoskeletal re-organization in response to Prolactin while downregulation of NEK3 expression by siRNA blocks these effects (2). Prolactin also stimulates interaction between NEK3 and paxillin leading to increased paxillin phosphorylation. Analysis of breast tissue microarrays show a significant up-regulation of NEK3 expression in malignant versus normal specimens.

1. Kimura, M. et al: Molecular cloning and characterization of the human NIMA-related protein kinase 3 gene (NEK3). Cytogenet Cell Genet. 2001;95(3-4):177-82.

2. Miller, S.L. et al: Nek3 kinase regulates prolactin-mediated cytoskeletal reorganization and motility of breast cancer cells. Oncogene. 2007 Jul 12;26(32):4668-78.

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.

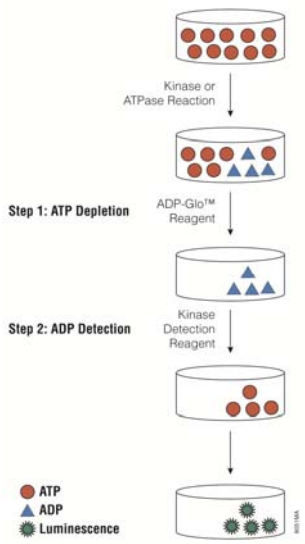

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

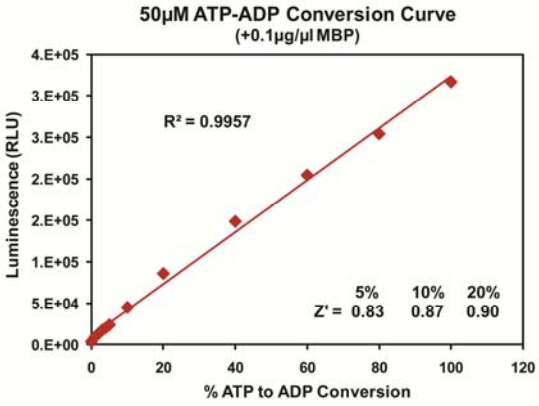

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase 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 60 minutes.
- 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 0.5-1sec).

Table 1. NEK3 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.

NEK3, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	295424	246333	162618	99848	57798	29203	15582	8311	4518	2790	1562
S/B	189	158	104	64	37	19	10	5	3	2	1
% Conversion	68	57	37	23	13	6	3	2	1	0.3	0

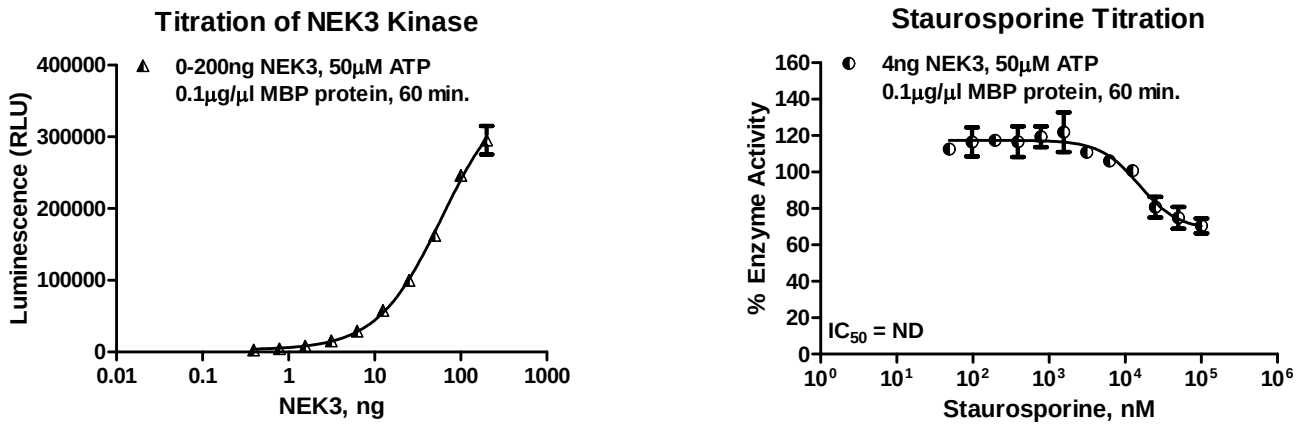

Figure 3. NEK3 Kinase Assay Development. **(A) NEK3 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 4ng of NEK3 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
NEK3 Kinase Enzyme System	Promega	V4500
ADP-Glo™ + NEK3 Kinase Enzyme System	Promega	V4501

NEK3 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT