

NEK2 Kinase Assay

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Scientific Background:

NEK2 is closely related in its catalytic domain to the serine/threonine protein kinase NIMA of *Aspergillus nidulans* that is required for entry into mitosis and may function in parallel to the universal mitotic inducer p34cdc2. Like NIMA, the NEK2 protein is almost undetectable during G1 but accumulated progressively throughout S, reaching maximal levels in late G2 (1). NEK2 is shown to be expressed most abundantly in the testis of the adult tissues examined being localized to the nucleus (2).

1. Schultz, SJ. et al: A. Cell cycle-dependent expression of Nek2, a novel human protein kinase related to the NIMA mitotic regulator of *Aspergillus nidulans*. *Cell Growth Differ.* 1994 Jun;5(6):625-35.
2. Fry, AM. et al: Substrate specificity and cell cycle regulation of the Nek2 protein kinase, a potential human homolog of the mitotic regulator NIMA of *Aspergillus nidulans*. *J Biol Chem.* 1995 May 26; 270(21):12899-905.

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.

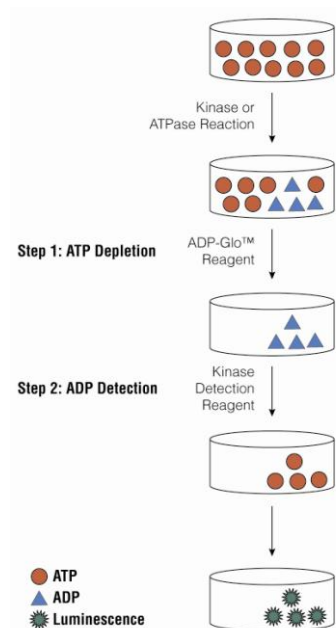


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

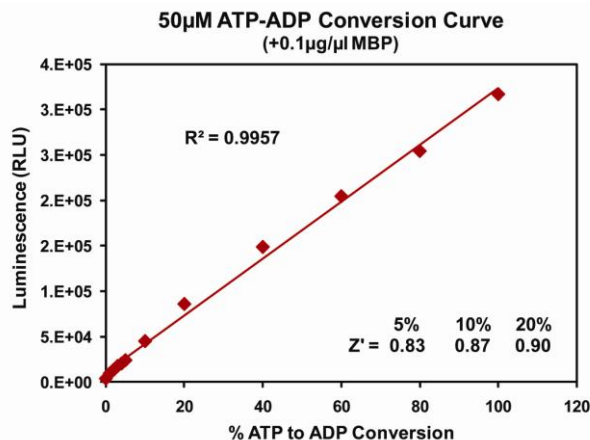


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
 - 1 μ l of inhibitor or (5% DMSO)
 - 2 μ l of enzyme (defined from table 1)
 - 2 μ l of substrate/ATP mix
- Incubate at room temperature for 30 minutes.
- Add 5 μ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. NEK2 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.

NEK2, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	128685	90765	67764	59684	33787	26008	15425	8840	6130	2889
S/B	45	31	23	21	12	9	5	3.1	2.1	1
% Conversion	71	49	35	31	15	11	4	2.2	1.2	0

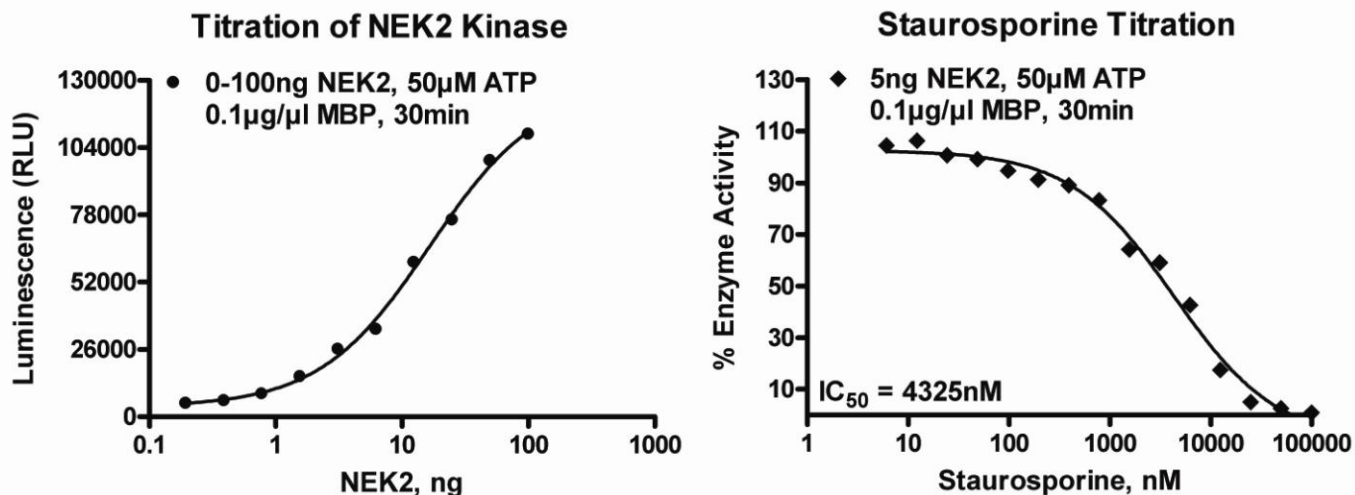


Figure 3. NEK2 Kinase Assay Development. (A) NEK2 enzyme was titrated using 50 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 5ng of NEK2 to determine the potency of the inhibitor (IC₅₀).

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
NEK2 Kinase Enzyme System	Promega	V3871
ADP-Glo™ + NEK2 Kinase Enzyme System	Promega	V9231

NEK2 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50 μ M DTT.

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