ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

NEK5 Kinase Assay

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Scientific Background:

NEK5 or NIMA (never in mitosis gene a)-related kinase 5 is a serine/threonine kinase that is involved in cell cycle regulation. NEK5 has been shown to interact with NEK6 and NEK5 are required for the ibo1/nek6 ectopic outgrowth phenotype in epidermal cells (1). NEK6 interacts and co-localizes with NEK5 in a transient expression assay. The ibo1-3 mutation markedly increases the interaction between NEK6 and NEK5. NEK6 forms heterodimers with NEK5 to regulate cortical microtubule organization possibly through the phosphorylation of β-tubulins. The NEK5 gene is conserved in chimpanzee, cow, mouse, rat, and chicken.

1. Motose, H. et al: NIMA-related kinases 6, 4, and 5 interact with each other to regulate microtubule organization during epidermal cell expansion in Arabidopsis thaliana. Plant J. 2011 Sep;67(6):993-1005.

2.

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.

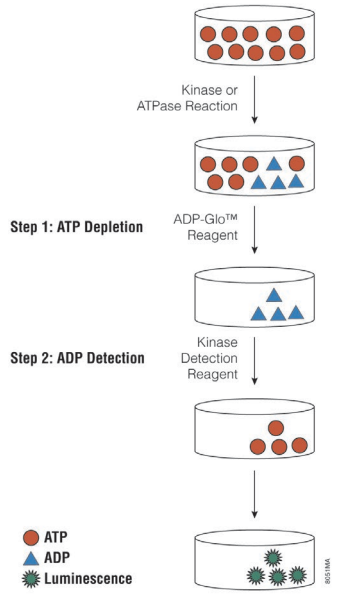

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

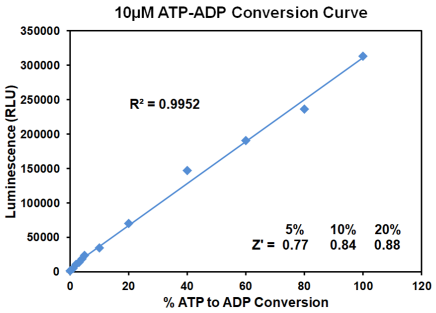

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	0
Luminescence	98,316	44,230	18,997	7,416	3,470	1,994	588
S/B	167	75	32	13	6	3	1
% Conversion	56	25	10	3	1	0	0

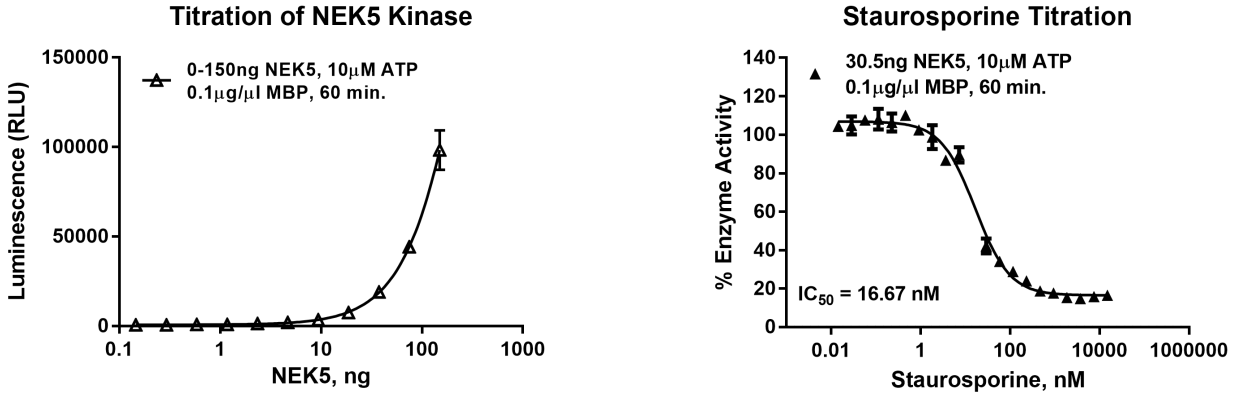

Figure 3. NEK5 Kinase Assay Development. (A) NEK5 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 30.5ng of NEK5 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
NEK5 Kinase Enzyme System	10µg	VA7246
	1mg	VA7247
ADP-Glo™ + NEK5 Kinase Enzyme System	1 Each	VA7248