ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

NUAK1 Kinase Assay

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Scientific Background:

NUAK1 or SNF1/AMP kinase-related kinase (SNARK) is a member of the NUAK family of SNF1-like kinase 1 that is also known as AMPK-related protein kinase 5 (ARK5). ARK5 is a tumor cell survival factor that is activated by AKT and acts as an ATM kinase under conditions of nutrient starvation (1). NUAK1 is highly expressed in heart and brain, and at lower levels in skeletal muscle, kidney, ovary, placenta, lung, and liver. NUAK1 is involved in tolerance to glucose starvation and suppresses Fas-induced apoptosis by phosphorylation of CASP6, thus suppressing the activation of the caspase and the subsequent cleavage of CFLAR (2).

1. Suzuki, A. et.al: Identification of a novel protein kinase mediating Akt survival signaling to the ATM protein. J. Biol. Chem. 278: 48-53, 2003.

2. Lizcano J.M. et.al: LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. EMBO J. 23:833-843, 2004.

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.

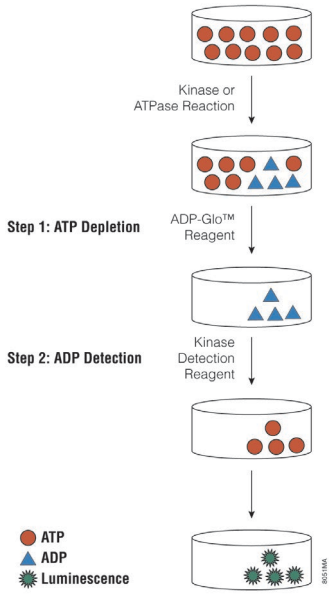

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

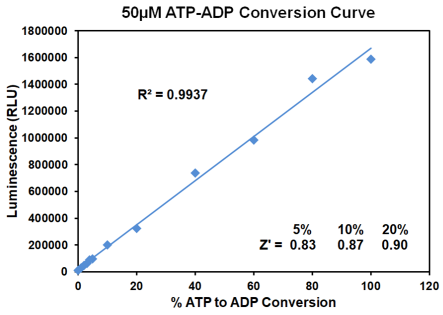

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	1,051,436	726,520	496,722	277,338	135,027	65,744	35,868	18,997	6,904
S/B	152	105	72	40	20	10	5	3	1
% Conversion	61	42	28	15	7	3	1	0	0

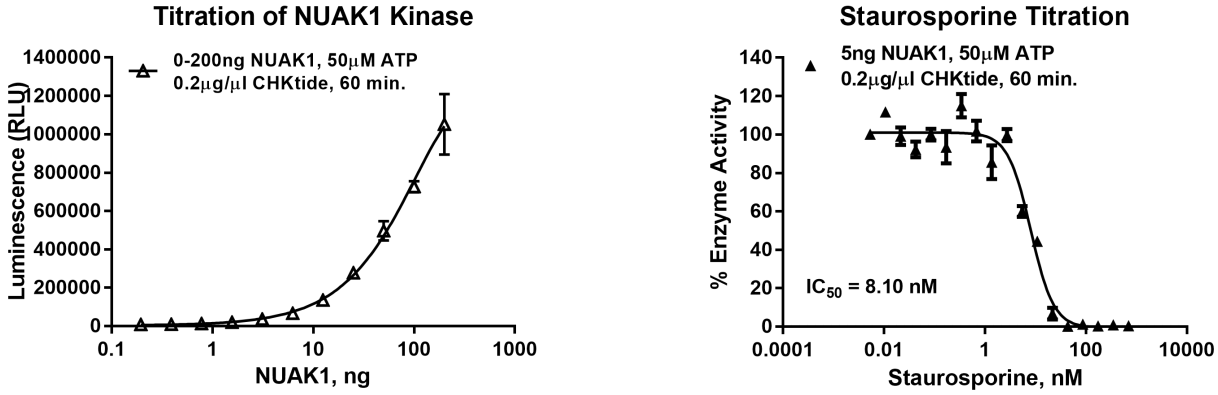

Figure 3. NUAK1 Kinase Assay Development. (A) NUAK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 5ng of NUAK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
NUAK1 Kinase Enzyme System	10µg	VA7252
	1mg	VA7253
ADP-Glo™ + NUAK1 Kinase Enzyme System	1 Each	VA7254