NUAK2 Kinase Assay

By Jacquelyn S. Turri, M.S., Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

NUAK2 or SNF1/AMP kinase-related kinase (SNARK) is a member of the NUAK family of SNF1-like kinase 2. NUAK2 is activated by muscle contraction and is a unique mediator of contraction-stimulated glucose transport in skeletal muscle (1). NUAK2 is involved in cellular stress responses linked to obesity and type 2 diabetes. NUAK2 shows kinase activity against a synthetic test peptide and the activity in keratinocytes is increased by AMP and 5-amino-4-imidazolecarboxamide riboside, implying that AMPK kinase-dependent pathway can activate NUAK2. Glucose deprivation also increases NUAK2 activity in baby hamster kidney fibroblasts (2).

1. Lefebvre, D. L. et.al: Identification and characterization of a novel sucrose-non-fermenting protein kinase/AMP-activated protein kinase-related protein kinase, SNARK. Biochem. J. 355: 297-305, 2001.

2. Rune, A. et al: Regulation of skeletal muscle sucrose, non-fermenting 1/AMP-activated protein kinase-related kinase (SNARK) by metabolic stress and diabetes. Diabetologia. 2009 Oct;52(10):2182-9.

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.

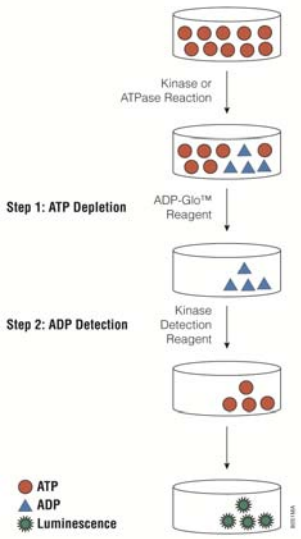

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The **ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.**

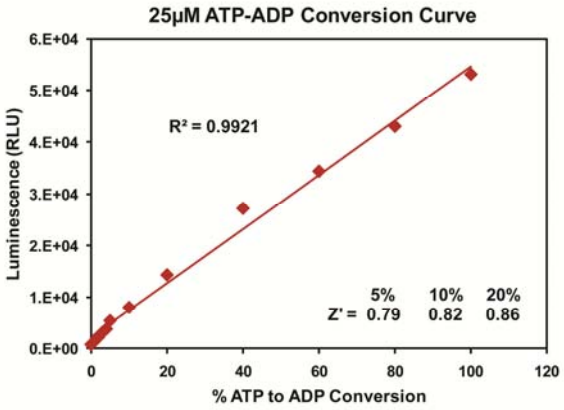

Figure 2. Linearity of the ADP-Glo Kinase Assay. **ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.**

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. NUAK2 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.

NUAK2, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
Luminescence	100734	66459	39792	22532	11696	5430	2957	1943	1680	928	676	416
S/B	242	160	96	54	28	13	7	5	4	2	2	1
% Conversion	49	33	19	11	5	2	1	0.8	0.6	0.3	0.2	0

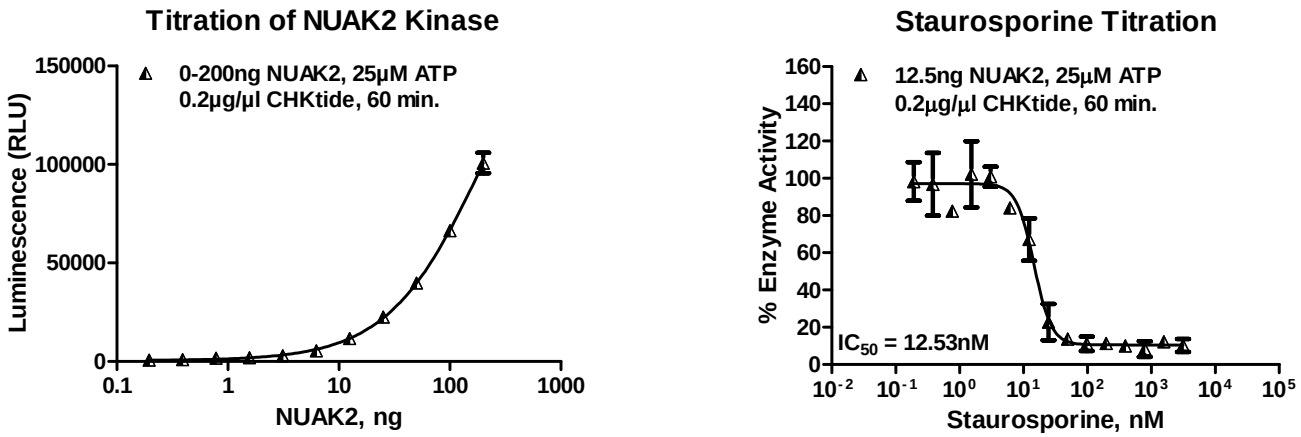

Figure 3. NUAK2 Kinase Assay Development. **(A) NUAK2 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 12.5ng of NUAK2 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
NUAK2 Kinase Enzyme System	Promega	V5096
ADP-Glo™ + NUAK2 Kinase Enzyme System	Promega	V5097

NUAK2 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.