EIF2AK2 Kinase Assay

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Scientific Background:

EIF2AK2 (also known as double-stranded RNA-activated protein kinase) is a protein kinase that has been shown to be involved in HIV/gp120-associated neurodegeneration (1). EIF2AK2 acts as a critical mediator of gp120 neurotoxicity and is a substrate for a family of protein kinases that respond to various forms of environmental stress. Activation of EIF2AK2 leads to its autophosphorylation and then phosphorylation of its natural substrate, the alpha subunit of eukaryotic protein synthesis initiation factor-2. EIF2AK2 plays a critical role in mRNA translation, cell proliferation and apoptosis. A cross-talk event between the EIF2AKs and p53 has been shown that has implications in cell proliferation and tumorigenesis (2).

1. Baltzis, D. et al: The eIF2alpha kinases PERK and PKR activate glycogen synthase kinase 3 to promote the proteasomal degradation of p53. J. Biol Chem. 2007; 282(43):31675-87
2. Alirezaei, M. et al: Human immunodeficiency virus-1/surface glycoprotein 120 induces apoptosis through RNA-activated protein kinase signaling in neurons. J. Neurosci. 2007;27(41):11047-55.

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.

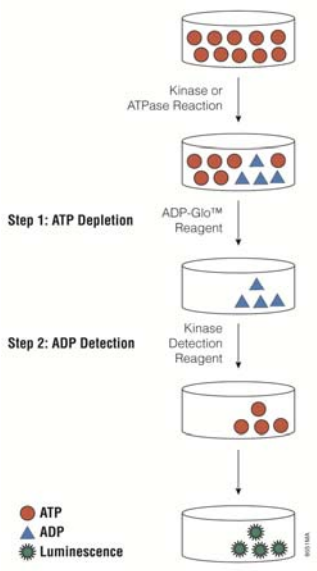

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

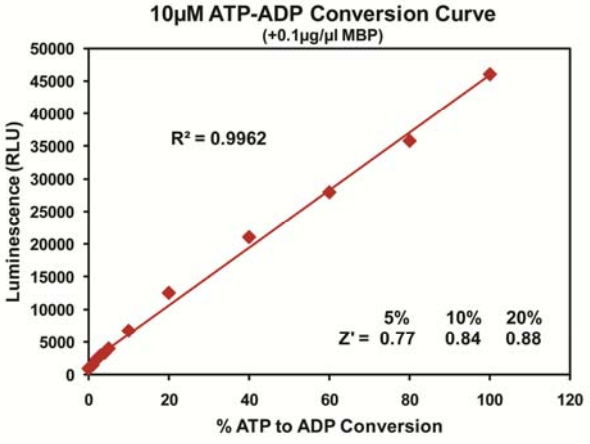

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. EIF2AK2 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.

EIF2AK2, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0
RLU	79007	66958	59192	49743	33534	17952	10199	5524	2876	281
S/B	281	238	211	177	119	64	36	20	10	1
% Conversion	108	91	79	65	42	22	12	7	3	0

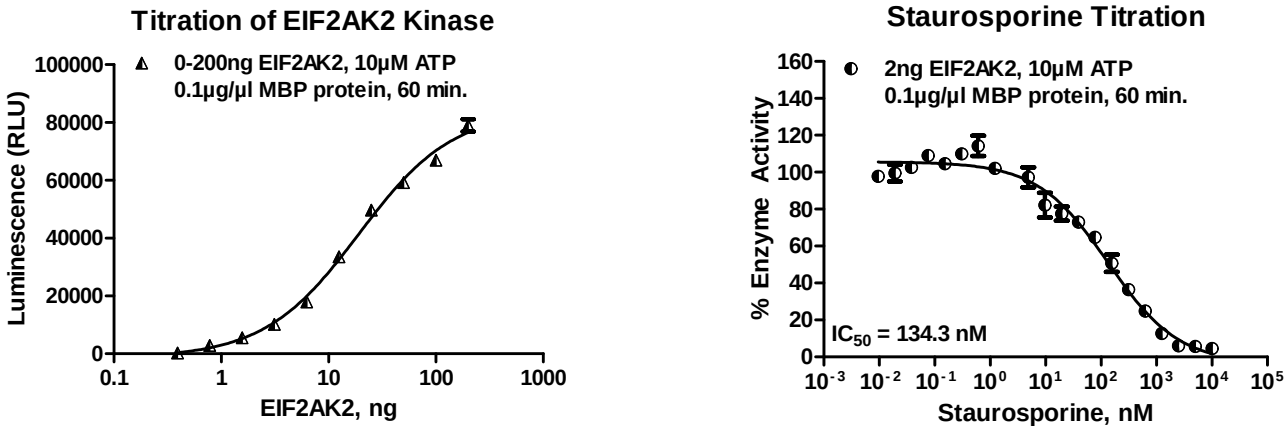

Figure 3. EIF2AK2 Kinase Assay Development. (A) EIF2AK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of EIF2AK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
EIF2AK2 Kinase Enzyme System	Promega	V5328
ADP-Glo™ + EIF2AK2 Kinase Enzyme System	Promega	V5329

EIF2AK2 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.