ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

EIF2AK4 Kinase Assay

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Scientific Background:

EIF2AK4 or eukaryotic translation initiation factor 2 alpha kinase 4 belongs to a family of kinases that phosphorylate the alpha subunit of eukaryotic translation initiation factor-2 to downregulate protein synthesis in response to varied cellular stresses (1). The EIF2AK4 eIF2-alpha kinase regulates fatty-acid homeostasis in the liver during deprivation of an essential amino acid (2).

1. Berlanga, J. J. et.al: Characterization of a mammalian homolog of the GCN2 eukaryotic initiation factor 2-alpha kinase. Europ. J. Biochem. 265: 754-762, 1999.

2. Guo, F. et.al: The GCN2 eIF2-alpha kinase regulates fatty-acid homeostasis in the liver during deprivation of an essential amino acid. Cell Metab. 5: 103-114, 2007.

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.

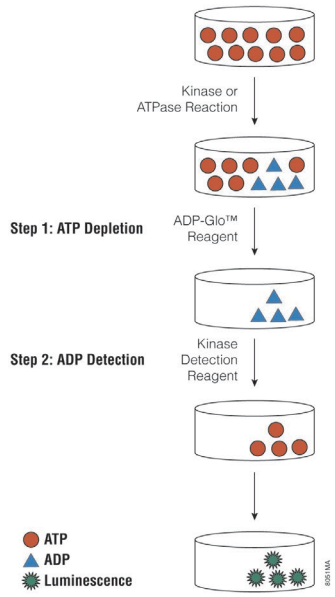

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

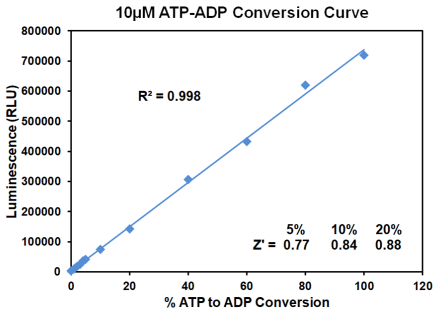

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	250	125	62.50	31.25	15.63	7.81	0
Luminescence	172,166	80,661	50,241	28,743	13,366	4,459	1,468
S/B	117	55	34	20	9	3	1
% Conversion	34	16	10	6	3	1	0

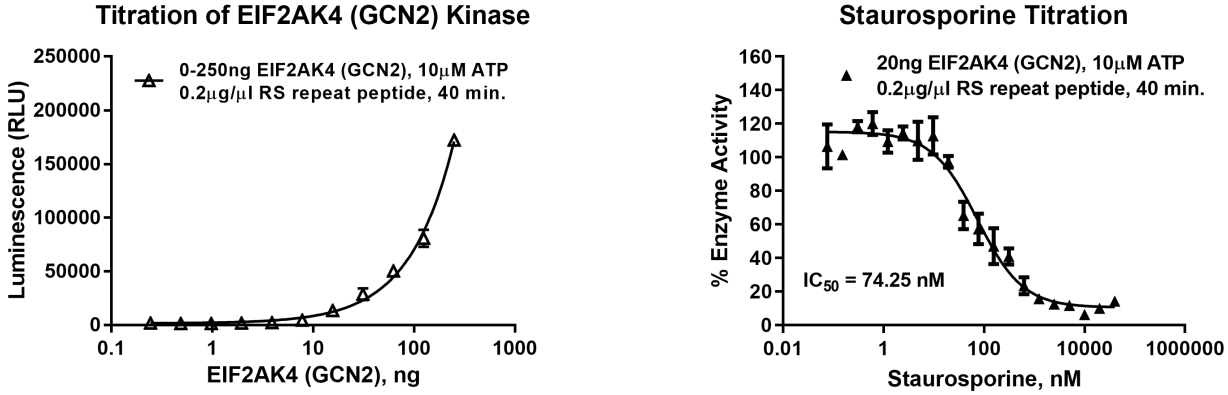

Figure 3. EIF2AK4 Kinase Assay Development. (A) EIF2AK4 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of EIF2AK4 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
EIF2AK4 Kinase Enzyme System	10µg	VA7438
	1mg	VA7439
ADP-Glo™ + EIF2AK4 Kinase Enzyme System	1 Each	VA7440