ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

AMPK (A1/B2/G2) Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

AMP-activated protein kinase (AMPK) exhibits a key role as a master regulator of cellular energy homeostasis (1). AMPK exists as a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits. Binding of AMP to the γ subunit allosterically activates the complex. AMPK is activated in response to stresses that deplete cellular ATP (low glucose, hypoxia and ischemia) (2) and via signaling pathways in response to adiponectin, leptin and CAMKKβ

1. Hardie, G.D. The AMP-activated protein kinase pathway – new players upstream and downstream. J. Cell Sci. 2004;117: 5479–5487.

2. Kahn, B.B. et al. AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab; 2005: 1, 15–25.

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.

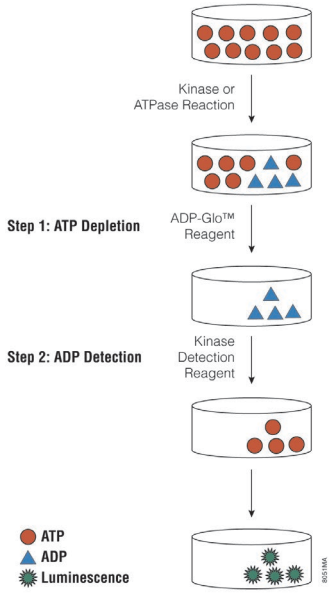

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

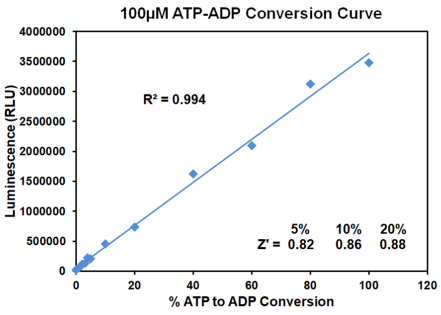

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0
Luminescence	1,705,510	1,555,345	1,510,655	1,358,190	1,124,070	731,952	419,595	214,468	123,385	67,000	22,633
S/B	75	69	67	60	50	32	19	9	5	3	1
% Conversion	46	42	41	36	30	19	10	4	2	0	0

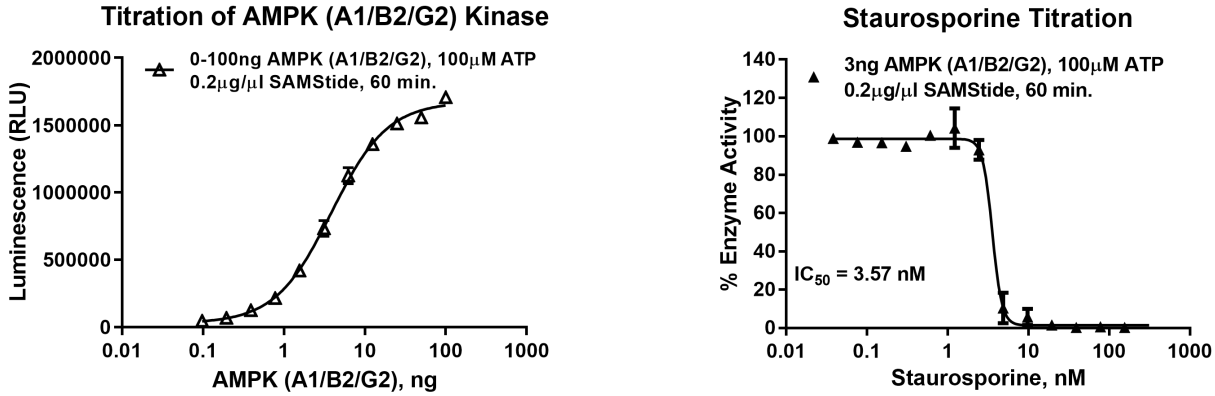

Figure 3. AMPK (A1/B2/G2) Kinase Assay Development. (A) AMPK (A1/B2/G2) enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of AMPK (A1/B2/G2) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
AMPK (A1/B2/G2) Kinase Enzyme System	10µg	VA7021
	1mg	VA7022
ADP-Glo™ + AMPK (A1/B2/G2) Kinase Enzyme System	1 Each	VA7023