ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: AMPK (A1/B1/G 2)

AMPK (A1/B1/G2) Kinase Assay

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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.

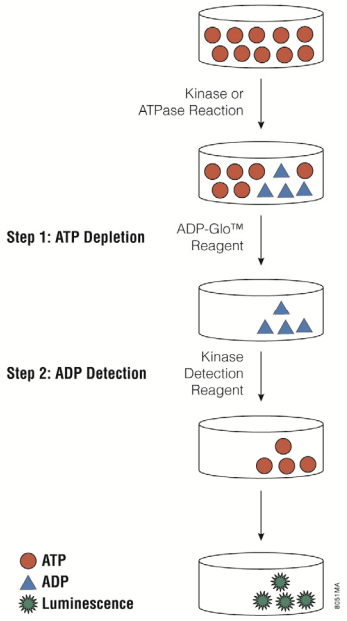


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

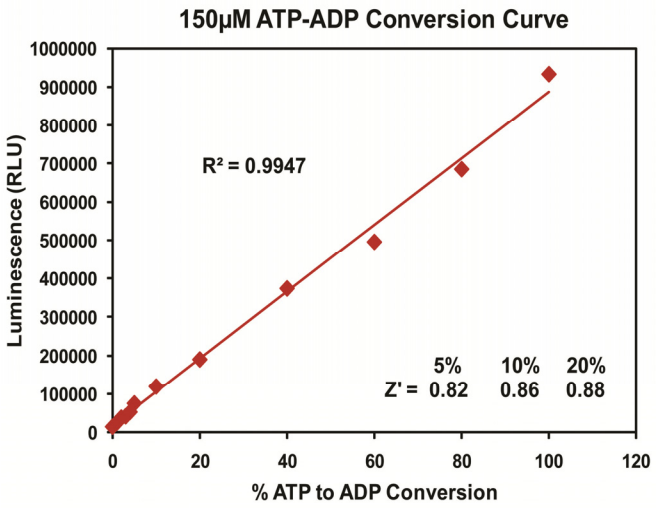


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 150µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

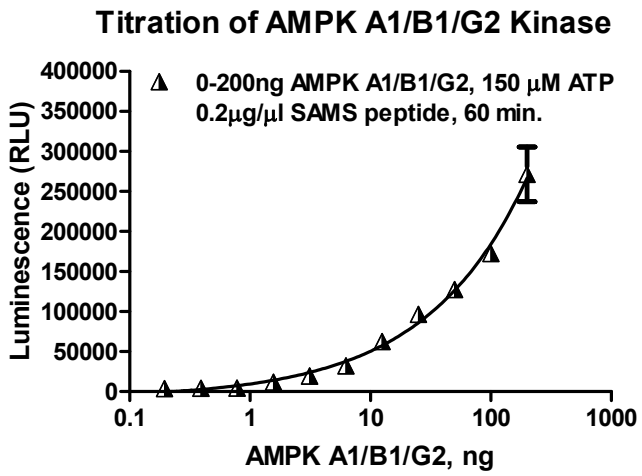
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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 0.5-1second).

Table 1. AMPK (A1/B1/G2) 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.

AMPK (A1/B1/G2), ng	200	100	50	25	13	6.3	3.1	1.6	0
RLU	271358	172574	127573	96577	62591	32272	19515	11891	2793
S/B	97	62	46	35	22	12	7	4.3	1
% Conversion	24	15	11	8	5	2.0	0.8	0.6	0



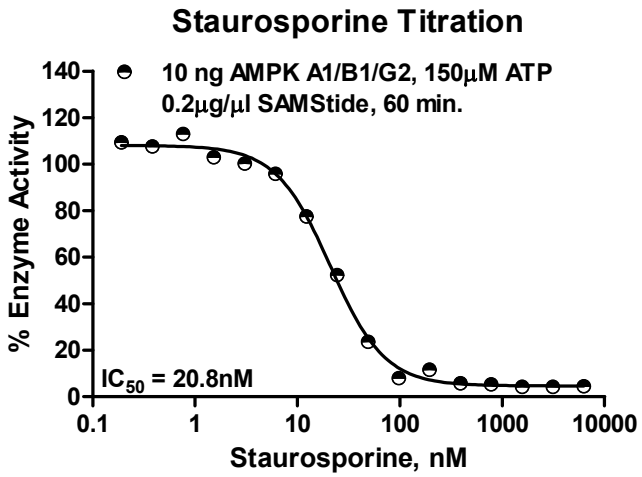


Figure 3. AMPK (A1/B1/G2) Kinase Assay Development. (A) AMPK (A1/B1/G2) enzyme was titrated using 150µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of AMPK (A1/B1/G2) to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
AMPK A1/B1/G2 Kinase Enzyme System	Promega	V4012
ADP-Glo™ + AMPK A1/B1/G2 Kinase Enzyme System	Promega	V4013

AMPK A1/B1/G2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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