ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

AMPK (A1/B2/G1) Kinase Assay

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

Scientific Background:

AMPK (A1/B2/G1) is a member of the AMPK family which are heterotrimeric proteins consisting of an alpha catalytic subunit, and non-catalytic beta and gamma subunits. AMPKs are an important energy-sensing enzyme group in the cells that monitor energy status particularly in response to stress (1). AMPKs regulate fatty acid and cholesterol synthesis by regulating the key rate-limiting enzymes acetyl-CoA carboxylase and hydroxy beta-methylglutaryl-CoA reductase. The β subunit may be a positive regulator of AMPK activity and is highly expressed in skeletal muscle (2).

1. Viollet, B. et al: Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. Biochem. Soc. Trans. 2003; 31; 216–219.

2. Thornton, C. et al: Identification of a novel AMP-activated protein kinase beta subunit isoform that is highly expressed in skeletal muscle. J. Biol. Chem. 273: 12443-12450, 1998.

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.

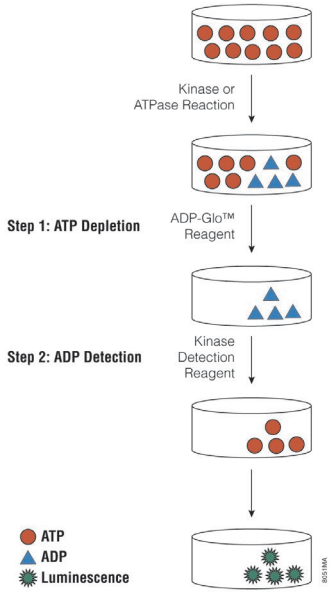

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

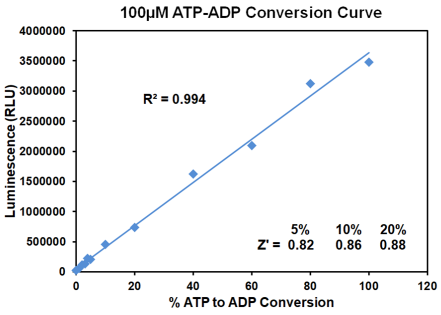

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

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	120	60	30	15	7.50	3.75	1.88	0.94	0.47	0.23	0
Luminescence	1,808,490	1,642,465	1,602,065	1,543,575	1,422,315	933,555	534,800	262,299	113,516	59,647	21,854
S/B	83	75	73	71	65	43	24	12	5	3	1
% Conversion	49	44	43	42	38	25	13	6	2	0	0

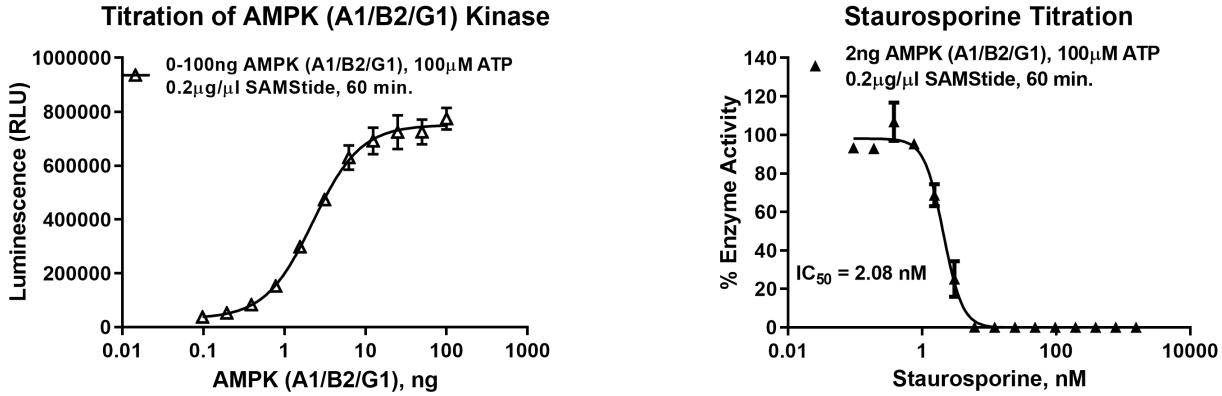

Figure 3. AMPK (A1/B2/G1) Kinase Assay Development. (A) AMPK (A1/B2/G1) enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of AMPK (A1/B2/G1) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
AMPK (A1/B2/G1) Kinase Enzyme System	10µg	VA7018
	1mg	VA7019
ADP-Glo™ + AMPK (A1/B2/G1) Kinase Enzyme System	1 Each	VA7020