ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

PHKG1 Kinase Assay

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Scientific Background:

PHKG1 or phosphorylase kinase gamma 1 is a member of the Ser/Thr protein kinase family that encodes a protein with one protein kinase domain and two calmodulin-binding domains. Phosphorylase kinase is a crucial glycogenolytic regulatory enzyme (1). PHKG1 is the catalytic member of a 16-subunit protein kinase complex that contains equimolar ratios of 4 subunit types known as alpha, beta, gamma and delta (2). Skeletal muscle contains the highest amount of phosphorylase kinase enzymatic activity, although activity is also observed in liver, cardiac muscle, brain, and several other tissues.

1. Burwinkel, B. et.al: Muscle glycogenosis with low phosphorylase kinase activity: mutations in PHKA1, PHKG1 or six other candidate genes explain only a minority of cases. Europ. J. Hum. Gen. 11: 516-526, 2003.

2. Wehner, M. et.al: Human cDNA encoding the muscle isoform of the phosphorylase kinase gamma subunit (PHKG1).

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.

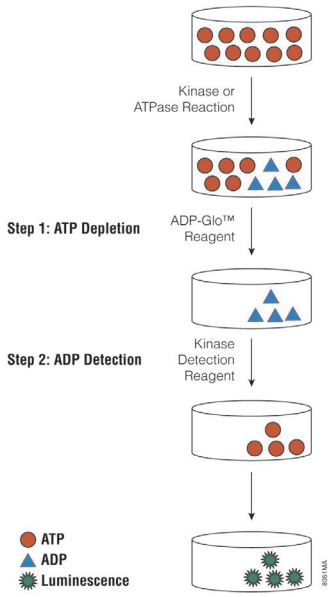

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

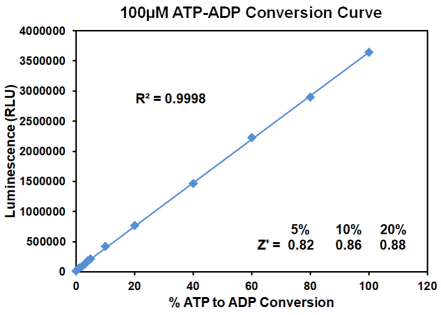

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	0
Luminescence	1,185,390	504,739	279,862	242,690	129,570	62,419	44,655	13,430
S/B	88	38	21	18	10	5	3	1
% Conversion	32	13	7	6	3	1	0	0

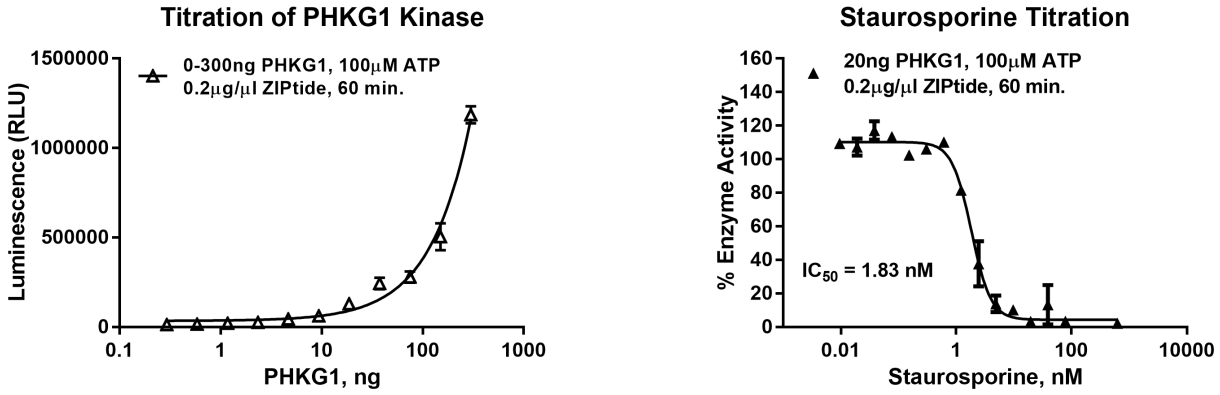

Figure 3. PHKG1 Kinase Assay Development. (A) PHKG1 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of PHKG1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PHKG1 Kinase Enzyme System	10µg	VA7528
	1mg	VA7529
ADP-Glo™ + PHKG1 Kinase Enzyme System	1 Each	VA7530