ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

CLK2 Kinase Assay

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

Scientific Background:

CDC-like kinase 2 (CLK2), a family of autophosphorylating kinases termed CLK (CDC2/CDC28-like kinases) was shown to phosphorylate SR proteins and to influence alternative splicing in overexpression systems (1). Recent findings demonstrated that the CLK kinases activate PTP-1B family members, and this phosphatase may be an important cellular target for CLK action. Mutations in the clk-2 proteins affect organismal features such as development, behavior, reproduction, and aging as well as cellular features such as the cell cycle, apoptosis, the DNA replication checkpoint, and telomere length (2).

1. Jiang, N. et al: Human CLK2 links cell cycle progression, apoptosis, and telomere length regulation. J. Biol. Chem. 2003;278(24):21678-84.

2. Nayler, O. et al: The cellular localization of the murine serine/arginine-rich protein kinase CLK2 is regulated by serine 141 autophosphorylation. J. Biol. Chem. 1998;273(51):34341-8.

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.

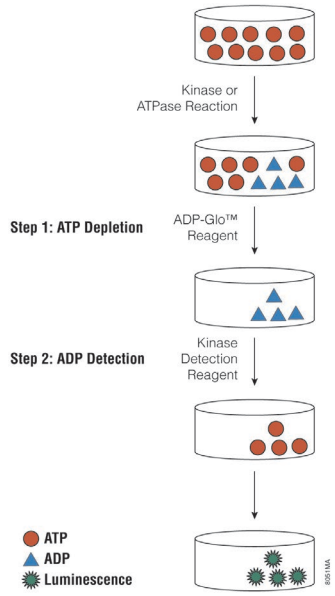

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

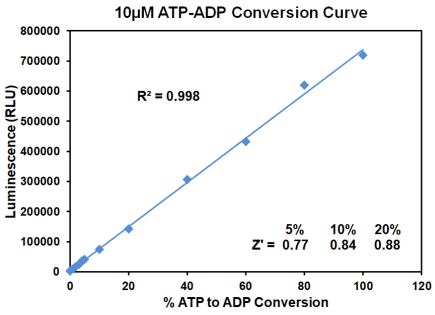

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0
Luminescence	201,112	197,697	178,328	172,418	145,762	90,328	61,148	29,765	17,261	9,240	6,223	1,440
S/B	140	137	124	120	101	63	42	21	12	6	4	1
% Conversion	62	61	55	53	45	27	18	8	4	2	1	0

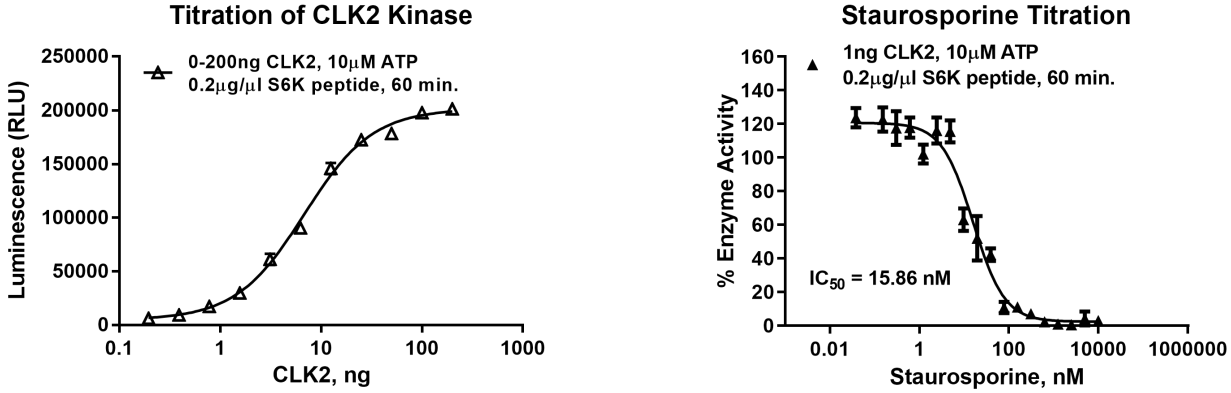

Figure 3. CLK2 Kinase Assay Development. (A) CLK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of CLK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CLK2 Kinase Enzyme System	10µg	VA7414
	1mg	VA7415
ADP-Glo™ + CLK2 Kinase Enzyme System	1 Each	VA7416