ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CLK1

CLK1 Kinase Assay

By Dongping Ma, M.S., Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

CLK/STY is a member of the CDC2-like (or LAMMER) family of dual specificity protein kinases. The phosphorylated serine/arginine-rich (SR) proteins involves in the pre-mRNA processing and releasing through nucleus into nucleoplasm. CLK/STY, which could phosphorylates the specific SR proteins (1), such as ASF/SF2, may directly regulates the activity and compartmentalization of SR splicing factors (2).

1. Prasad J et al: Regulation and substrate specificity of the SR protein kinase Clk/Sty. Mol Cell Biol. 2003 Jun; 23(12): 4139-49.

2. Colwill, K. et al: SRPK1 and Clk/Sty protein kinases show distinct substrate specificities for serine/arginine-rich splicing factors. J. Biol. Chem. 271: 24569-24575, 1996.

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.

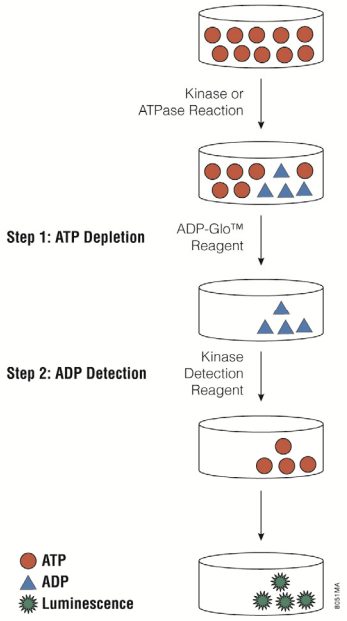


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

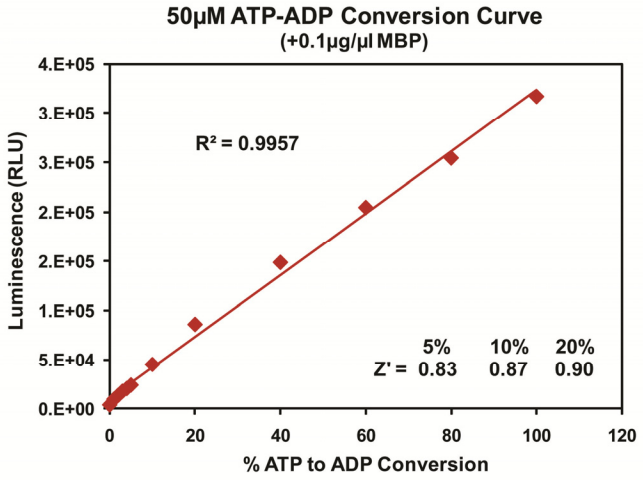


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

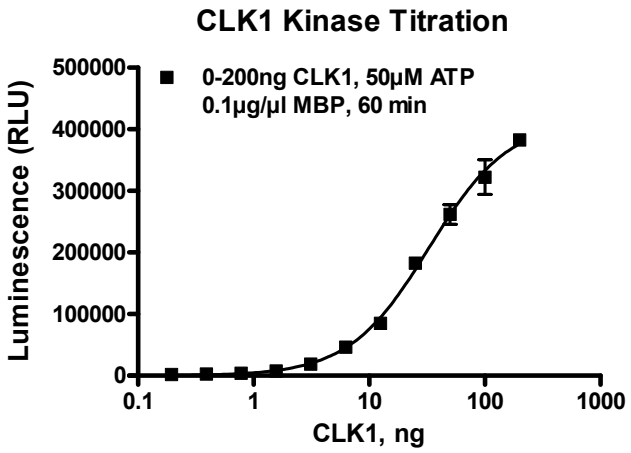
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. CLK1 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.

CLK1, ng	200	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0
RLU	377474	322451	261787	182730	85332	46485	19083	7787	3806	2712	1265
S/B	298	255	207	144	67	37	15	6	3	2	1
% Conversion	100	85	69	48	22	12	5	2	0.8	0.5	0



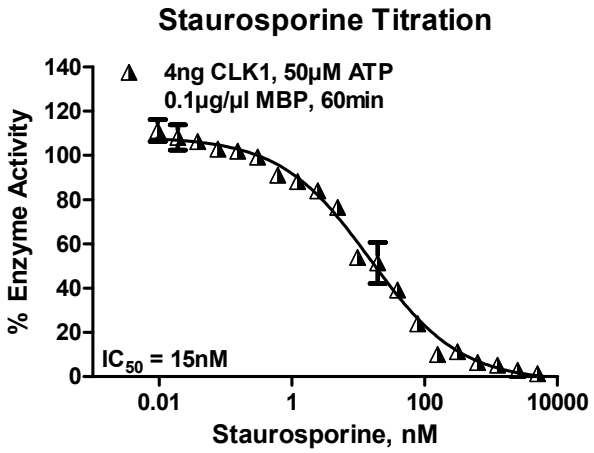


Figure 3. CLK1 Kinase Assay Development. **(A) CLK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 4ng of CLK1 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CLK1 Kinase Enzyme System	Promega	V4056
ADP-Glo™ + CLK1 Kinase Enzyme System	Promega	V4057

CLK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.