ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CLK3

CLK3 Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

CLK3, also known as CDC-like kinase 3, encodes a serine/threonine type protein kinase with a non-conserved N-terminal domain. A long and short isoform (phclk3 and pclk3/152) result from alternative splicing and coexist in different tissues (1). The CLK3 protein has the molecular functions of ATP binding, nucleotide binding, protein serine/threonine kinase activity, protein-tyrosine kinase activity and transferase activity and the CLK3 protein localize in both the cytoplasm and nuclear compartment. CLK3 is thought to regulate the intranuclear distribution of the serine/arginine-rich (SR) family of splicing factors.

1. Hanes, J. et al: , Characterization by cDNA cloning of two new human protein kinases. Evidence by sequence comparison of a new family of mammalian protein kinases". J. Mol. Biol. 1994; 244 (5): 665–72.

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.

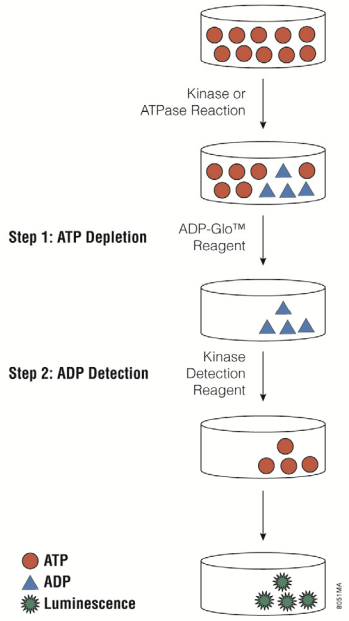


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

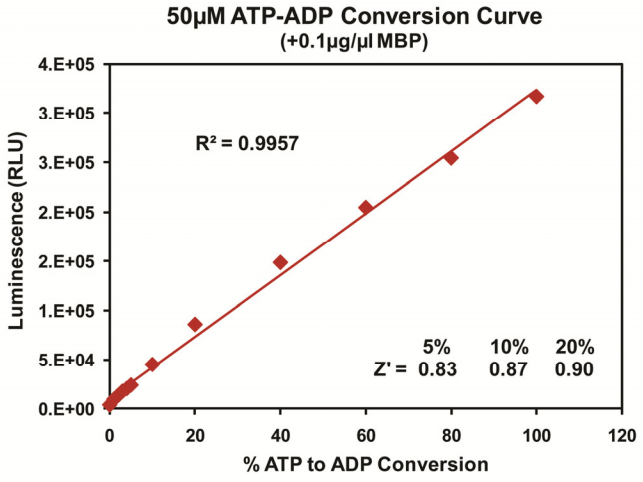


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

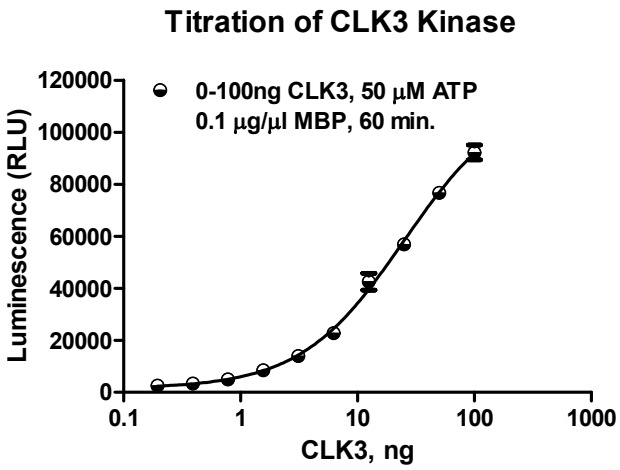
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. CLK3 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.

CLK3, ng	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0
RLU	92195	76609	56801	42504	22733	13957	8443	4997	3388	1696
S/B	54	45	33	25	13	8	5	3	2	1
% Conversion	30	24	17	12	4	2.2	1.4	0.6	0.2	0



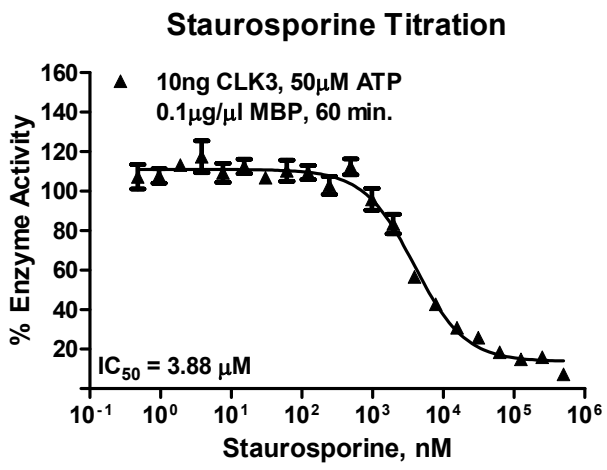


Figure 3. CLK3 Kinase Assay Development. (A) CLK3 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of CLK3 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CLK3 Kinase Enzyme System	Promega	V4162
ADP-Glo™ + CLK3 Kinase Enzyme System	Promega	V4163

CLK3 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
promega.com