ADP-Glo™ Kinase Assay Application Note

Ser-Thr Kinase Series

CLK4 Kinase Assay

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Scientific Background:

CLK4 or CDC-like kinase 4 belongs to the CDC2-like protein kinase (CLK) family that can interact with and phosphorylate the serine- and arginine-rich (SR) proteins. The SR proteins are known to play an important role in the formation of spliceosomes and thus may be involved in the regulation of alternative splicing. CLK4 expressed in E. coli phosphorylated myelin basic protein as a substrate as well as autophosphorylating itself but it did not phosphorylate H2B (1).

1. Schultz, J.et.al: Molecular characterization of a cDNA encoding functional human CLK4 kinase and localization to chromosome 4q35.

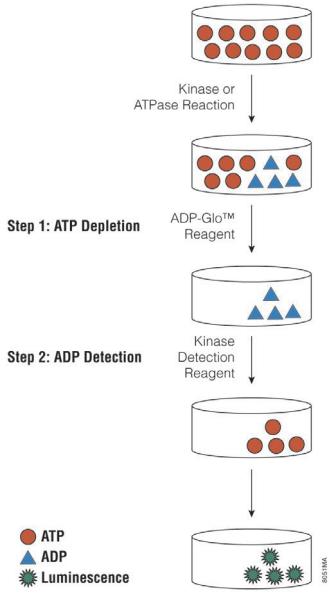

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

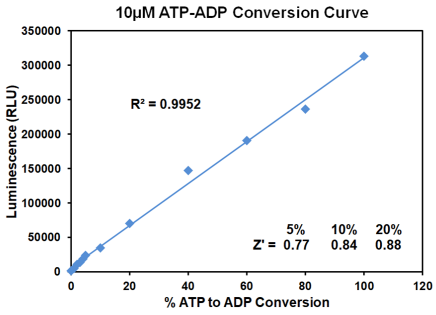

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0
Luminescence	181,755	105,275	68,577	36,315	22,665	10,239	6,585	3,384	2,310	1,323	629
S/B	289	167	109	58	36	16	10	5	4	2	1
% Conversion	104	59	38	19	11	4	2	0	0	0	0

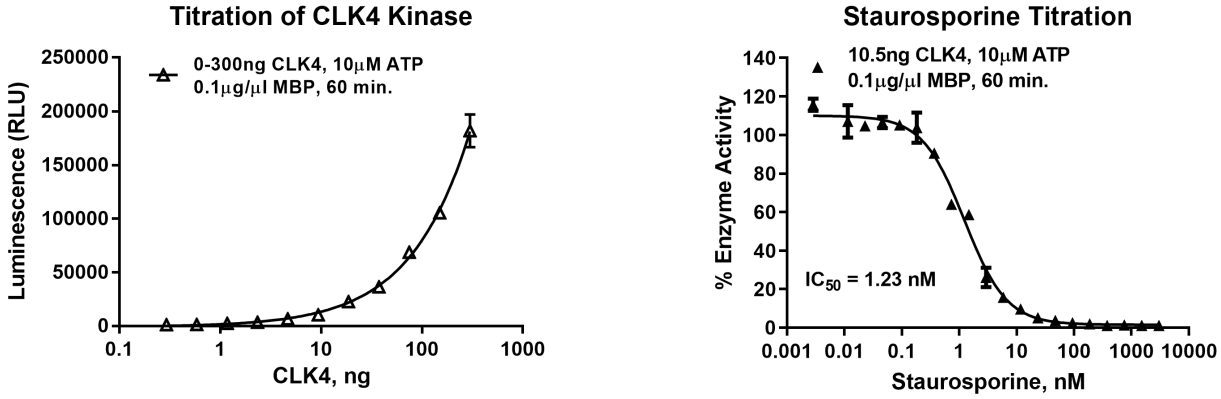

Figure 3. CLK4 Kinase Assay Development. (A) CLK4 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 10.5ng of CLK4 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CLK4 Kinase Enzyme System	10µg	VA7072
	1mg	VA7073
ADP-Glo™ + CLK4 Kinase Enzyme System	1 Each	VA7074