ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

CDK1/CyclinA1 Kinase Assay

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

Scientific Background:

CDK1 or Cell Division Control protein 1 is essential for the completion of START, the controlling event in the cell cycle that is required to initiate mitosis. CDK1 is a catalytic subunit of a protein kinase complex, called the M-Phase Promoting Factor that induces entry into mitosis and is universal among eukaryotes (1). Phosphorylation of Bcl-2 in G2/M phase-arrested cells following photodynamic therapy with hypericin involves a CDK1-mediated signal and delays the onset of apoptosis. Therapeutic potential of CDK inhibitor NU2058 in androgen-independent prostate cancer has also been demonstrated (2).

1. Vantieghem, A. et al: Phosphorylation of Bcl-2 in G2/M phase-arrested cells following photodynamic therapy with hypericin involves a CDK1-mediated signal and delays the onset of apoptosis. J. Biol. Chem. 2002; 277(40):37718-31.

2. Rigas, A.C. et al: Therapeutic potential of CDK inhibitor NU2058 in androgen-independent prostate cancer. Oncogene. 2007; 18.

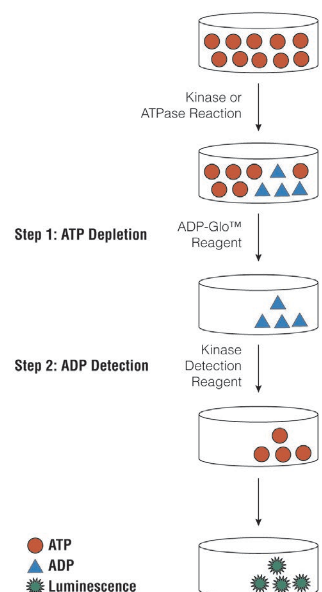

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

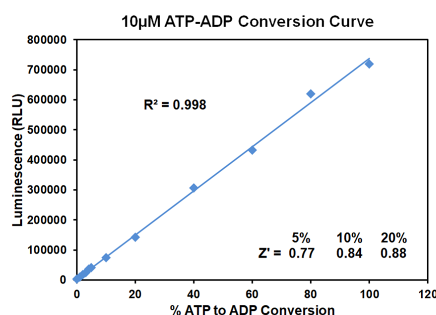

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

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	0.29	0
Luminescence	320,696	276,001	272,927	208,470	166,878	106,823	66,736	34,371	23,270	14,659	9,933	4,733
S/B	68	58	58	44	35	23	14	7	5	3	2	1
% Conversion	74	64	63	48	38	24	15	8	5	3	2	0

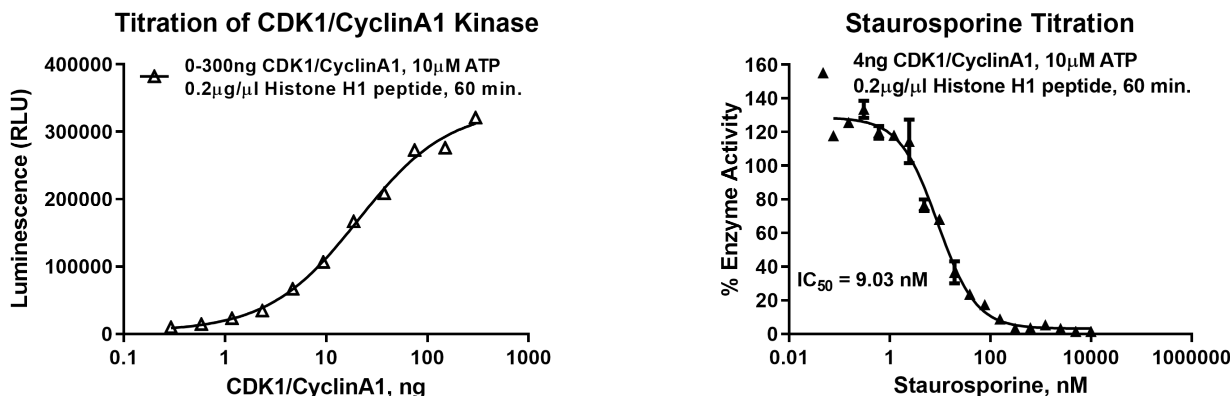

Figure 3. CDK1/CyclinA1 Kinase Assay Development. (A) CDK1/CyclinA1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 4ng of CDK1/CyclinA1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CDK1/CyclinA1 Kinase Enzyme System	10µg	VA7399
	1mg	VA7400
ADP-Glo™ + CDK1/CyclinA1 Kinase Enzyme System	1 Each	VA7401