ADP-Glo™ Kinase Assay Application Notes

SER-THR SERIES: CDK1/CyclinA2

CDK1/CyclinA2 Kinase Assay

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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.

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.

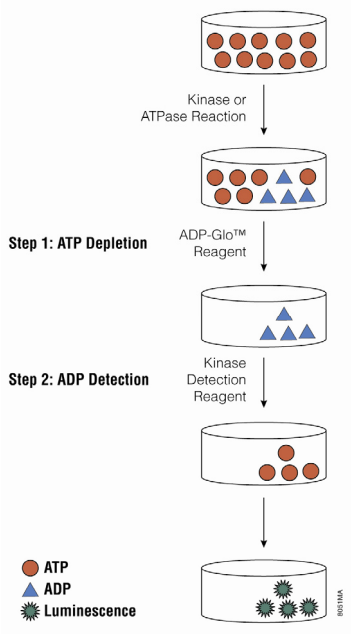

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

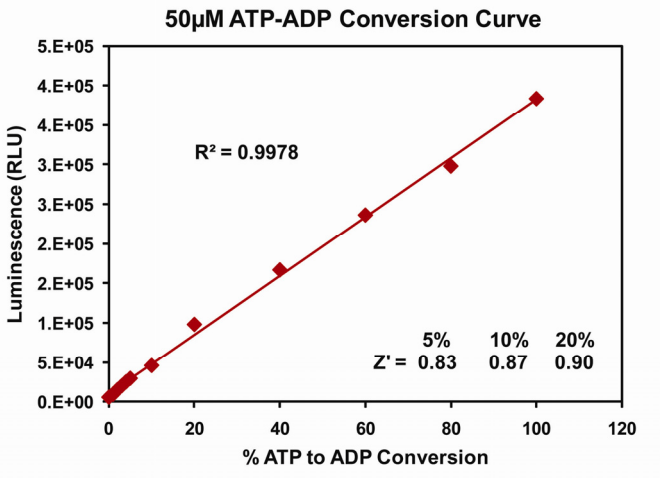

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. CDK1/CyclinA2 Enzyme Titration. **Reactions were carried out for 60 minutes and kinase activity was determined using ADP-Glo. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. Correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.**

CDK1, ng	25	12.5	6.25	3.12	1.5	0.78	0.39	0.2	0
RLU	155175	124816	107732	77604	47652	28776	16947	11235	3277
S/B	47.4	38.1	32.9	23.7	14.5	8.8	5.2	3.4	1
% Conversion	54.7	43.5	37.2	26.1	15.1	8.1	3.8	1.7	0

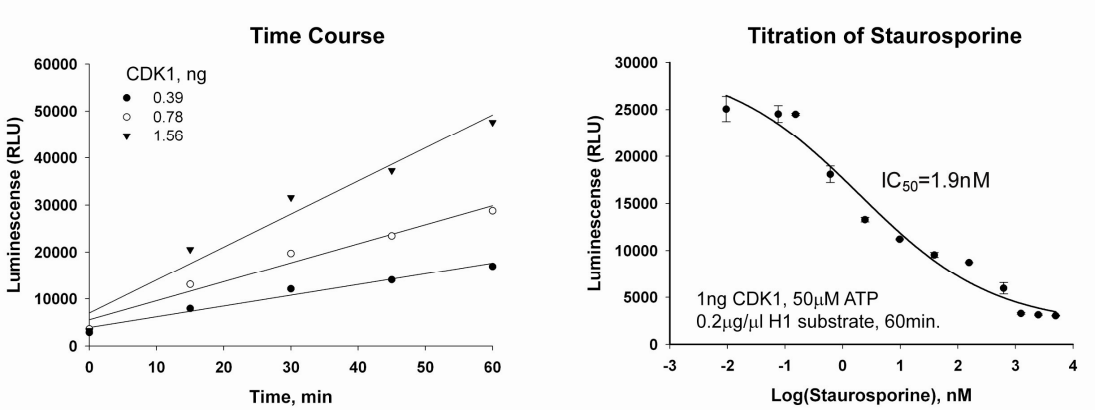

Figure3. CDK1/CyclinA2 Kinase Assay Development. **CDK1/CyclinA2 linear response curves were obtained at indicated amounts of enzyme using 0.2µg/µl of Histone H1 substrate and 50µM ATP. To determine the potency of the inhibitor (IC_{50}) staurosporine dose response was performed under conditions indicated in the figure.**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CDK1/CyclinA2 Kinase Enzyme System	Promega	V2961
ADP-Glo + CDK1/CyclinA2 Kinase Enzyme System	Promega	V9211

CDK1/CyclinA2 Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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