ADP-Glo™ Kinase Assay Application Notes

SER-THR SERIES: CDK2/CyclinA2

CDK2/CyclinA2 Kinase Assay

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Scientific Background:

CDK2 is a member of the Cyclin-Dependent Kinase family that is ubiquitously expressed. CDK2 is a catalytic subunit of the cyclin-dependent protein kinase complex, whose activity is restricted to the G1-S phase, and essential for cell cycle G1/S phase transition. CDK2 associates with and is regulated by the regulatory subunits of the complex including Cyclin A or E, CDK inhibitor p21Cip1 (CDKN1A) and p27Kip1 (CDKN1B) (1). CDK2 phosphorylates multiple cellular substrates including SMAD3 and FOXO1. Phosphorylation of FOXO1 leads to its inhibition (2).

1. Levkau, B. et al: Cleavage of p21(Cip1/Waf1) and p27(Kip1) mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. Molec. Cell1: 553-563, 1998.
2. Huang, H. et al: CDK2-dependent phosphorylation of FOXO1 as an apoptotic response to DNA damage. Science314: 294-297, 2006.

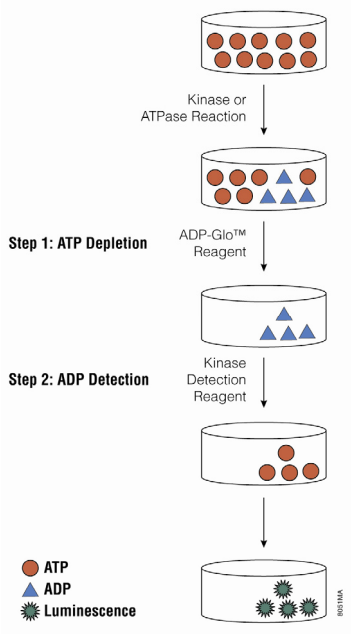

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

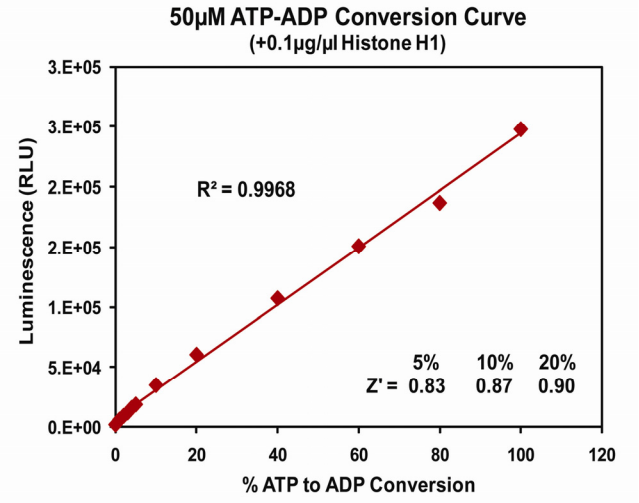

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 10 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. CDK2/Cyclin A2 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.

CDK2/Cyclin A2, ng	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	130351	119747	113541	100345	89972	73778	51645	33838	22546	4275
S/B	30	28	27	23	21	17	12	8	5	1
% Conversion	53	48	46	40	35	28	18	10	5	0

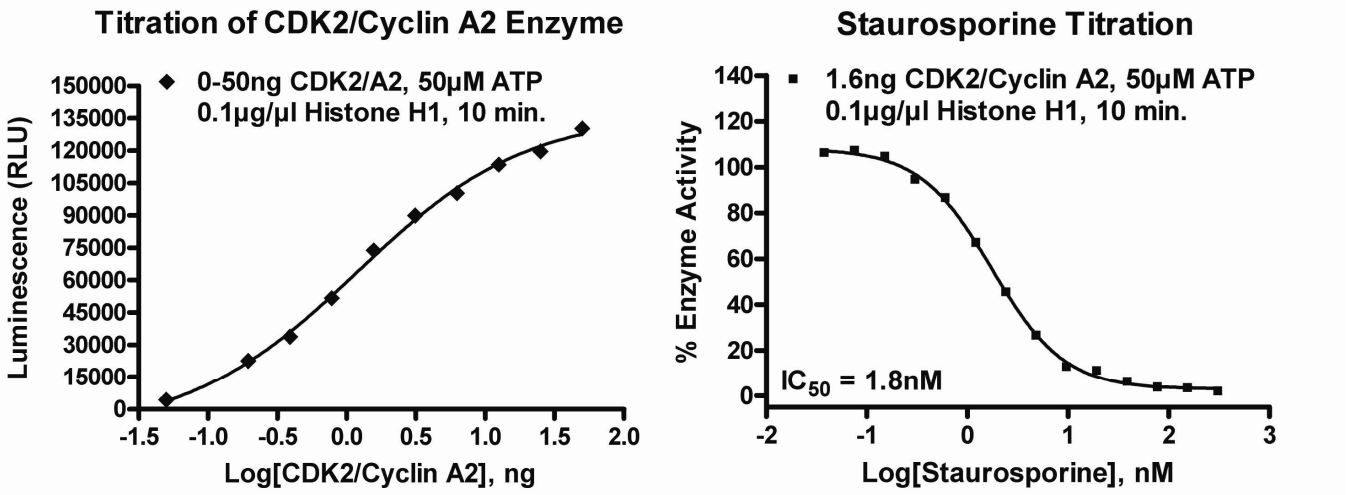

Figure 2. CDK2/Cyclin A2 Kinase Assay Development. (A) CDK2/Cyclin A2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1.6ng of CDK2/Cyclin A2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CDK2/CyclinA2 Kinase Enzyme System	Promega	V2971
ADP-Glo + CDK2/CyclinA2 Kinase Enzyme System	Promega	V9221

CDK2/Cyclin A2 Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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