ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

CDK2/Cyclin E2 Kinase Assay

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

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. Cell 1: 553-563, 1998.

2. Huang, H. et al: CDK2-dependent phosphorylation of FOXO1 as an apoptotic response to DNA damage. Science 314: 294-297, 2006.

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.

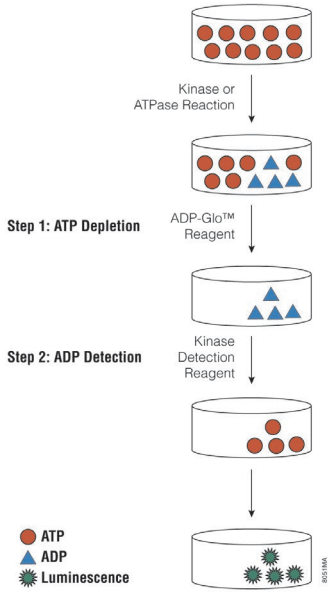

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

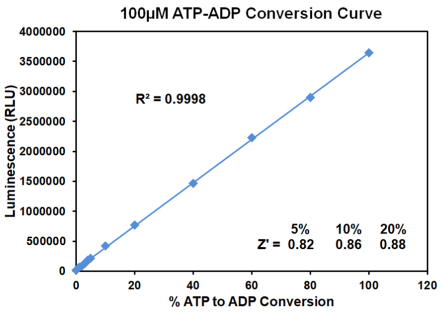

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0
Luminescence	2,030,305	1,356,585	951,251	507,099	251,188	110,205	55,844	29,054	6,507
S/B	312	208	146	78	39	17	9	4	1
% Conversion	79	53	37	19	9	4	1	0	0

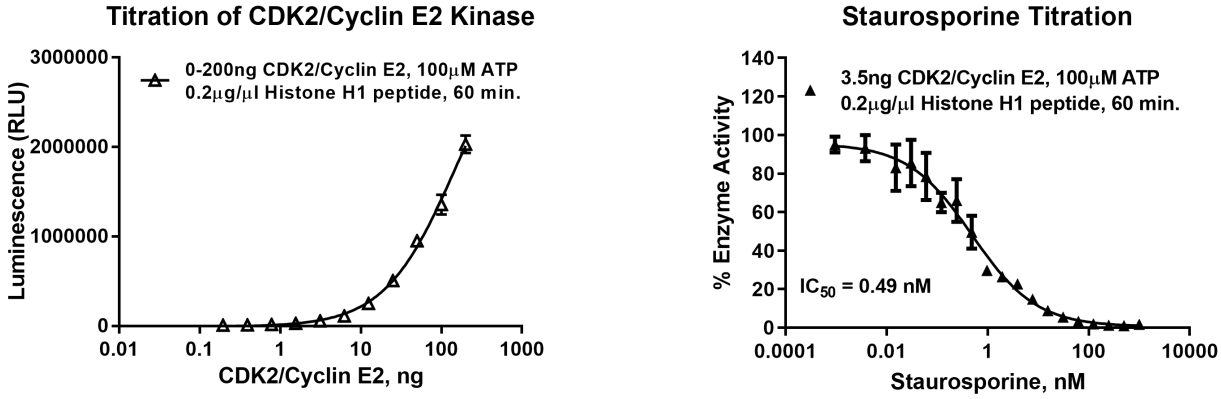

Figure 3. CDK2/Cyclin E2 Kinase Assay Development. (A) CDK2/Cyclin E2 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3.5ng of CDK2/Cyclin E2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CDK2/Cyclin E2 Kinase Enzyme System	10µg	VA7042
	1mg	VA7043
ADP-Glo™ + CDK2/Cyclin E2 Kinase Enzyme System	1 Each	VA7044