

## CDK2/Cyclin A1 Kinase Assay

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### Scientific Background:

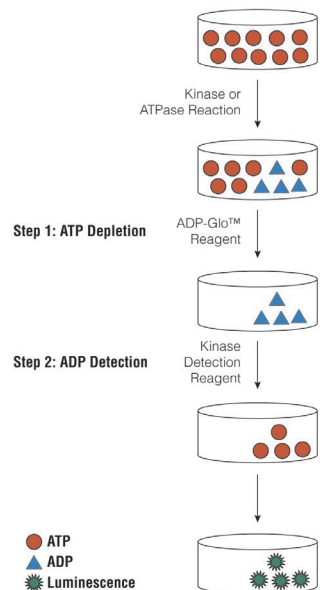
CDK2 is a member of the Cyclin-Dependent Kinase family that is ubiquitously expressed. CDK2 is a catalytic subunit of the cyclin-dependent protein kinase complex, whose activity is restricted to the G1-S phase, and essential for cell cycle G1/S phase transition. CDK2 associates with and is regulated 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. 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.

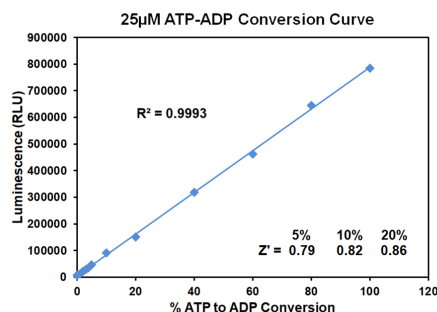
### ADP-Glo™ Kinase Assay

#### 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 25µM 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

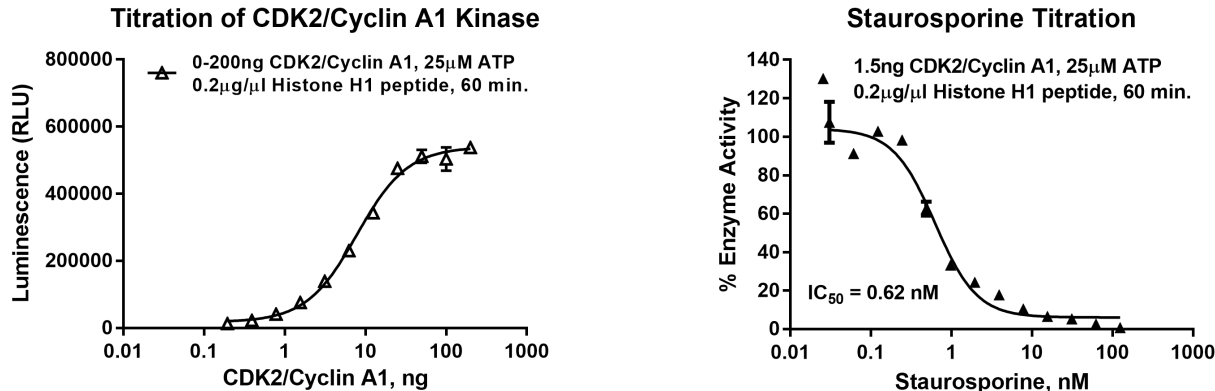
The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: <http://www.promega.com/KESProtocol>

## Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
  - ✓ 1  $\mu$ l of inhibitor or (5% DMSO)
  - ✓ 2  $\mu$ l of enzyme (defined from table 1)
  - ✓ 2  $\mu$ l of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5  $\mu$ l of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10  $\mu$ l 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	0.78	0.39	0.20	0
Luminescence	537,483	503,661	511,972	475,696	343,250	230,347	139,098	75,781	40,869	22,532	12,878	2,005
S/B	268	251	255	237	171	115	69	38	20	11	6	1
% Conversion	84	79	80	75	54	36	21	11	6	3	1	0



**Figure 3. CDK2/Cyclin A1 Kinase Assay Development.** (A) CDK2/Cyclin A1 enzyme was titrated using 25 $\mu$ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1.5ng of CDK2/Cyclin A1 to determine the potency of the inhibitor ( $IC_{50}$ ).



## Ordering Information:

Products	Size	Cat. #
CDK2/Cyclin A1 Kinase Enzyme System	10 $\mu$ g	VA7039
	1mg	VA7040
ADP-Glo™ + CDK2/Cyclin A1 Kinase Enzyme System	1 Each	VA7041