

# CDK6/CyclinD3 Kinase Assay

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## Scientific Background:

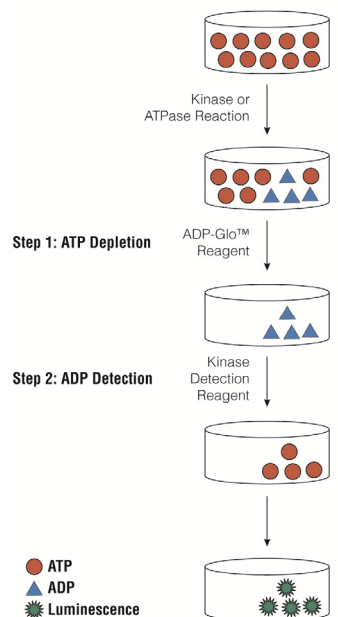
CDK6 is a member of the cyclin-dependent family of protein kinases that are important regulators of cell cycle progression. CDK6 activity is regulated by the D-type cyclins and members of the INK4 family of CDK inhibitors (1). The CDK6 kinase activity is detected in mid-G1 phase of the cell cycle and is responsible for the phosphorylation and regulation of the activity of tumor suppressor protein Rb. Although CDK6 and CDK4 can both phosphorylate multiple residues in the Rb protein, they do so with different residue selectivities *in vitro*; CDK6 phosphorylates Thr821 while CDK4 phosphorylates Thr826 on Rb protein (2).

1. Meyerson, M. et al: Identification of G1 kinase activity for cdk6, a novel cyclin D partner. *Molec. Cell. Biol.* 14: 2077-2086, 1994.
2. Takaki, T. et al: Preferences for phosphorylation sites in the retinoblastoma protein of D-type cyclin-dependent kinases, Cdk4 and Cdk6, *in vitro*. *J Biochem.* 2005 Mar;137(3):381-6.

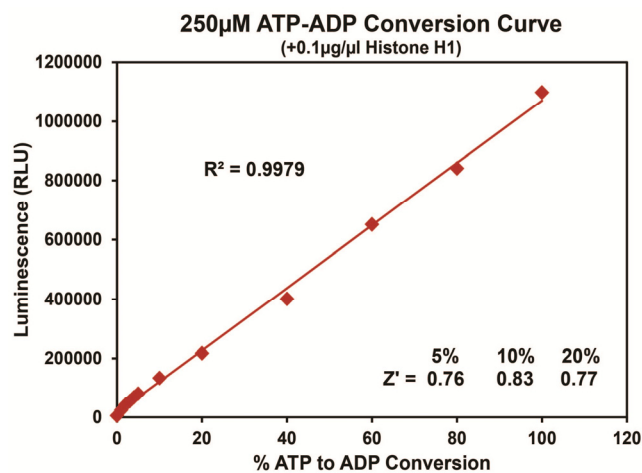
## ADP-Glo™ Kinase Assay

### 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 250µM 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](http://www.promega.com/tbs/tm313/tm313.html)

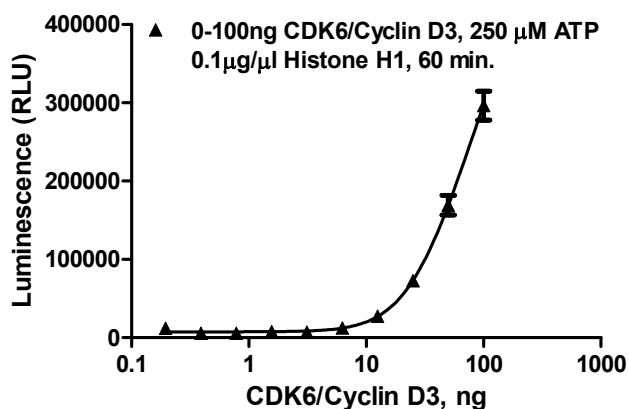
## Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1  $\mu$ 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 60 minutes.
- 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 0.5-1second).

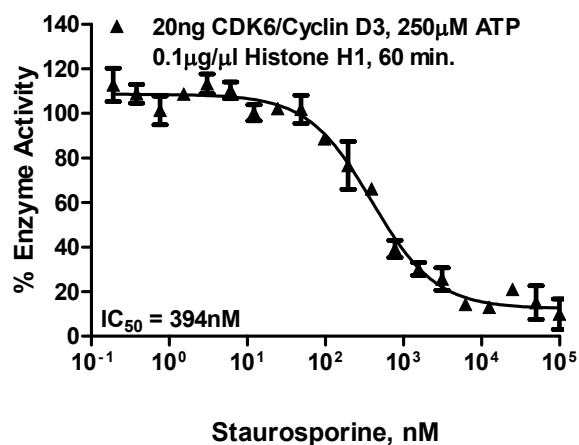
**Table 1. CDK6/CyclinD3 Enzyme Titration.** Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

CDK6/CyclinD3, ng	200	100	50	25	13	6.3	3.1	0
RLU	885779	296450	169291	72676	27640	12436	7223	4709
S/B	188	63	36	15	6	2.6	1.5	1
% Conversion	82	27	15	5	1.2	0.5	0.3	0

### Titration of CDK6/Cyclin D3 Kinase



### Staurosporine Titration



**Figure 3. CDK6/CyclinD3 Kinase Assay Development.** (A) CDK6/CyclinD3 enzyme was titrated using 250 $\mu$ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 20ng of CDK6/CyclinD3 to determine the potency of the inhibitor ( $IC_{50}$ ).

Assay Components and Ordering Information:		Promega	SignalChem Specialists in Signaling Proteins
Products	Company	Cat.#	
ADP-Glo™ Kinase Assay	Promega	V9101	
CDK6/CyclinD3 Kinase Enzyme System	Promega	V4510	
ADP-Glo™ + CDK6/CyclinD3 Kinase Enzyme System	Promega	V4511	

CDK6/CyclinD3 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl<sub>2</sub>; 0.1mg/ml BSA; 50 $\mu$ M DTT.