ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CDK9/CyclinK

CDK9/CyclinK Kinase Assay

By Juliano Alves, Ph.D., Mary Sobol, M.S., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

CDK9/CyclinK is a member of the cyclin-dependent protein kinase (CDK) family. CDK9 is closely related to cdc28 and cdc2 and is an important regulator of the cell cycle (1). CDK9 is a component of the multiprotein complex TAK/P-TEFβ. CDK9 can modulate RNA polymerase II-directed transcription by phosphorylating the C-terminal domain of the largest subunit of RNA polymerase II. CDK9 forms a complex with and is regulated by its regulatory subunit cyclin T or cyclin K. CDK9 also interacts with the HIV-1 Tat protein which suggested a possible involvement of this protein in AIDS (2).

1. Yang, Z. et al: The 7SK small nuclear RNA inhibits the CDK9/cyclin T1 kinase to control transcription. Nature, 2001; 414: 317-322.

2. Bullrich, F. et al: Chromosomal mapping of members of the cdc2 family of protein kinases, cdk3, cdk6, PISSLRE, and PITALRE, and a cdk inhibitor, p27-Kip1, to regions involved in human cancer. Cancer Res. 1995; 55: 1199-1205.

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.

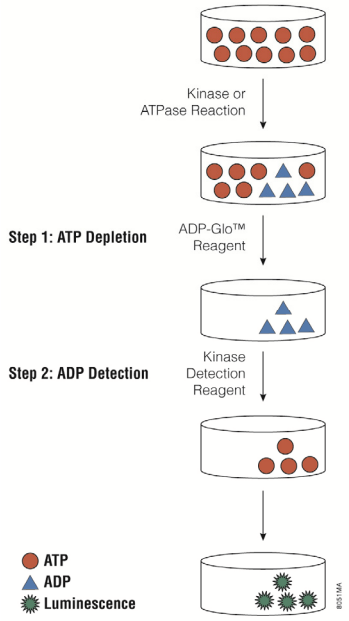

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

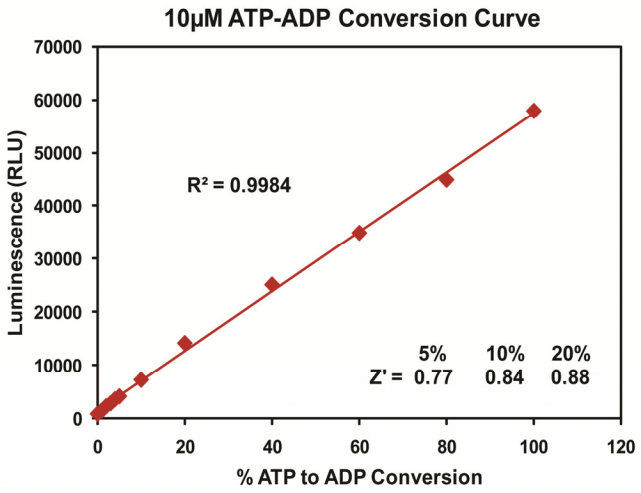

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 120 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. CDK9/CyclinK 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.

CDK9/CyclinK, ng	200	100	50	25	13	6.3	3.1	0
RLU	36853	16938	7190	2720	1589	827	548	269
S/B	137	63	27	10	6	3	2	1
% Conversion	53	24	10	3	2	1	0.27	0

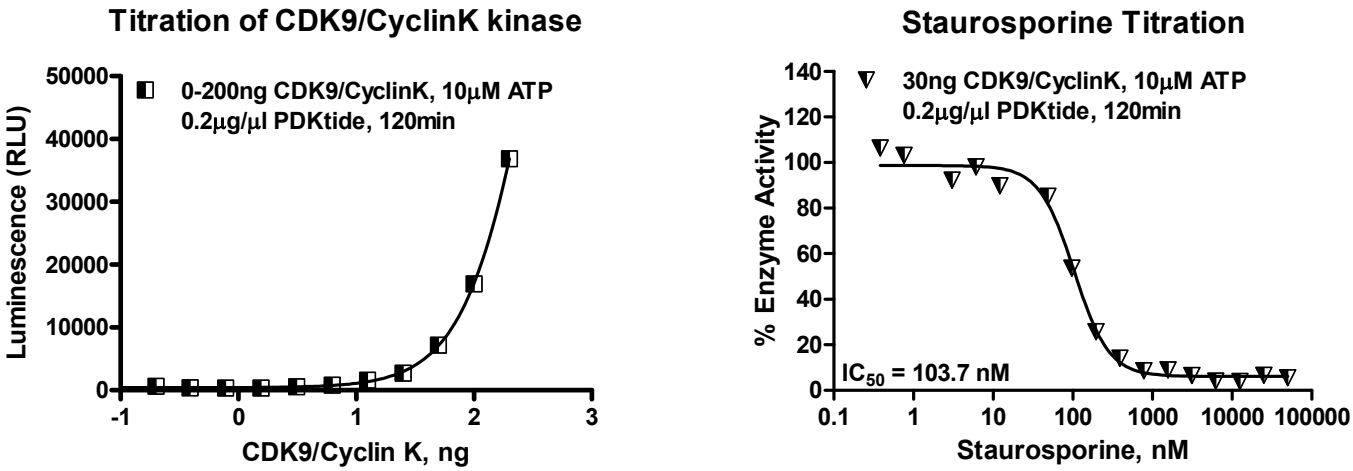

Figure 3. CDK9/CyclinK Kinase Assay Development. (A) CDK9/CyclinK enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 30ng of CDK9/CyclinK to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CDK9/CyclinK Kinase Enzyme System	Promega	V4104
ADP-Glo™ + CDK9/CyclinK Kinase Enzyme System	Promega	V4105

CDK9/CyclinK Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.