ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CDK3/CyclinE1

CDK3/CyclinE1 Kinase Assay

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

Scientific Background:

CDK3/CyclinE1is a member of the cyclin-dependent protein kinase family and promotes entry into S phase, in part by activating members of the E2F family of transcription factors. CDK3 can also associate with cyclin C and phosphorylates the retinoblastoma 1 protein to promote exit from G0. The CDK3 gene has been mapped to chromosomal location of 17q22-qter, telomeric to the BRCA1 gene by somatic cell hybrids analysis (1). The presence of a single point mutation in the CDK3 gene from several Mus musculus strains commonly used in the laboratory has been reported (2).

1. 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. 55: 1199-1205, 1995.

2. Ye, X. et al: A premature-termination mutation in the Mus musculus cyclin-dependent kinase 3 gene. Proc. Nat. Acad. Sci. 98: 1682-1686, 2001.

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.

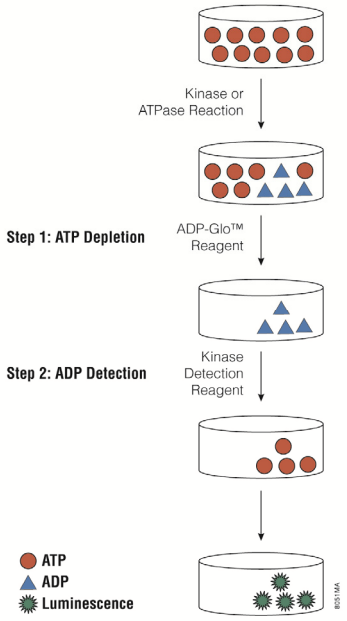

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

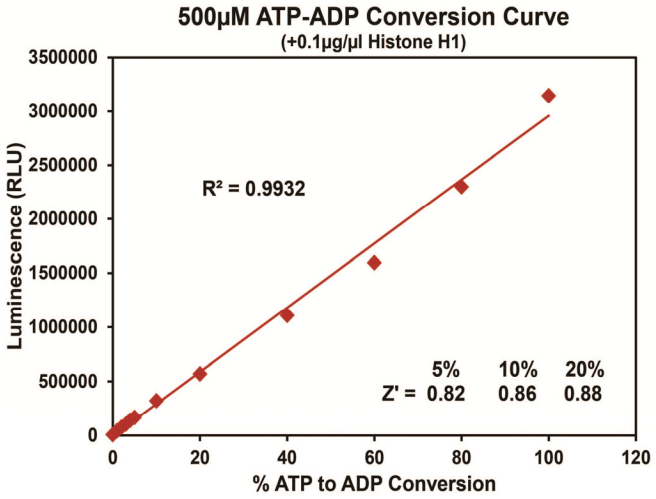

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 500µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 60 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. CDK3/CyclinE1 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.

CDK3/CyclinE1, ng	50	25	13	6.3	3.1	1.6	0.8	0
RLU	138000	107479	86354	61937	43814	27593	18713	7372
S/B	19	15	12	8	6	4	3	1
% Conversion	5	4	3	2.2	1.6	1.1	0.8	0

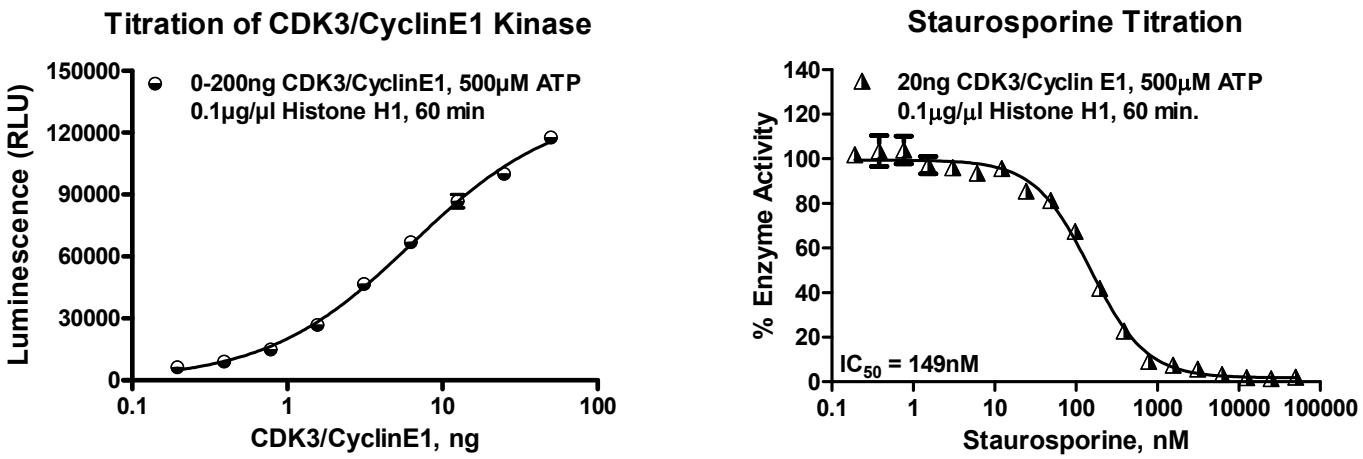

Figure 3. CDK3/CyclinE1 Kinase Assay Development. (A) CDK3/CyclinE1 enzyme was titrated using 500µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 20ng of CDK3/CyclinE1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CDK3/CyclinE1 Kinase Enzyme System	Promega	V4490
ADP-Glo™ + CDK3/CyclinE1 Kinase Enzyme System	Promega	V4491

CDK3/CyclinE1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.