ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CHK1

CHK1 Kinase Assay

By Jolanta Vidugiriene, Ph.D., Hicham Zegzouti, Ph.D., Gediminas Vidugiris, Ph.D., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

CHK1 is a 56 kd serine/threonine protein kinase that was originally identified in fission yeast to play a role in activation of the DNA damage checkpoint in the G2 phase of the cell cycle (1). CHK1 appears to function downstream of several of the known fission yeast checkpoint gene products, including that encoded by rad3+, a gene with sequence similarity to the ATM gene mutated in patients with ataxia telangiectasia (2).

1. Walworth, N. et al: Fission yeast CHK1 protein kinase links the rad checkpoint pathway to cdc2. Nature. 1993 May 27;363(6427):368-71.
2. Walworth, NC. et al: Rad-dependent response of the CHK1-encoded protein kinase at the DNA damage checkpoint. Science. 1996 Jan 19;271(5247):353-6. 1. Walworth, N. et al: Fission yeast CHK1 protein kinase links the rad checkpoint pathway to cdc2. Nature. 1993 May 27;363(6427):368-71.

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.

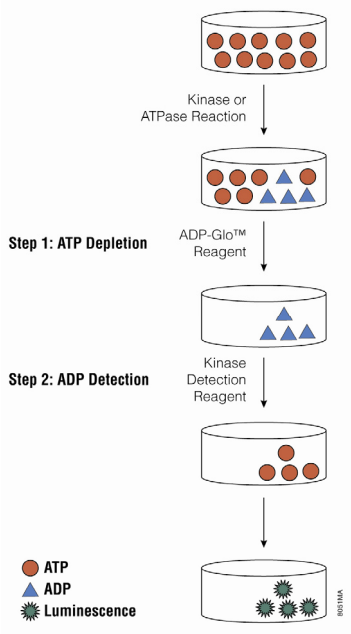

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

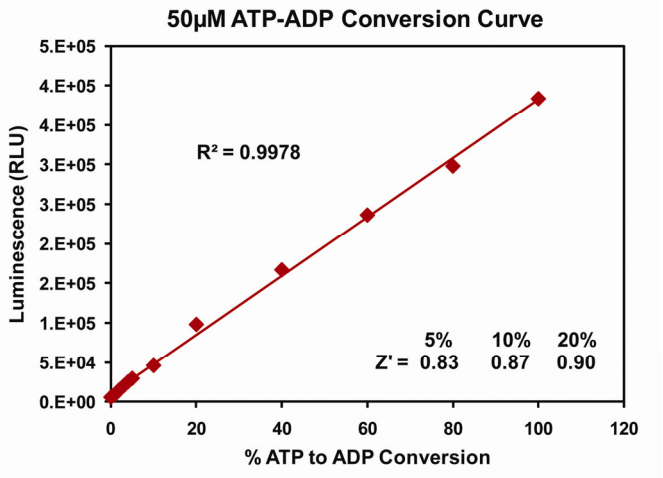

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. CHK1 Enzyme Titration. Reactions were carried out for 60 minutes and kinase activity was determined using ADP-Glo. 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.

CHK1, ng	50	25	12.5	6.25	3.12	1.56	0.78	0
RLU	126999	95240	62088	34503	19936	11105	7441	3872
S/B	32.799	24.597	16.035	8.9109	5.1486	2.868	1.922	1
% Conversion	44.681	32.984	20.773	10.613	5.2473	1.995	0.645	0

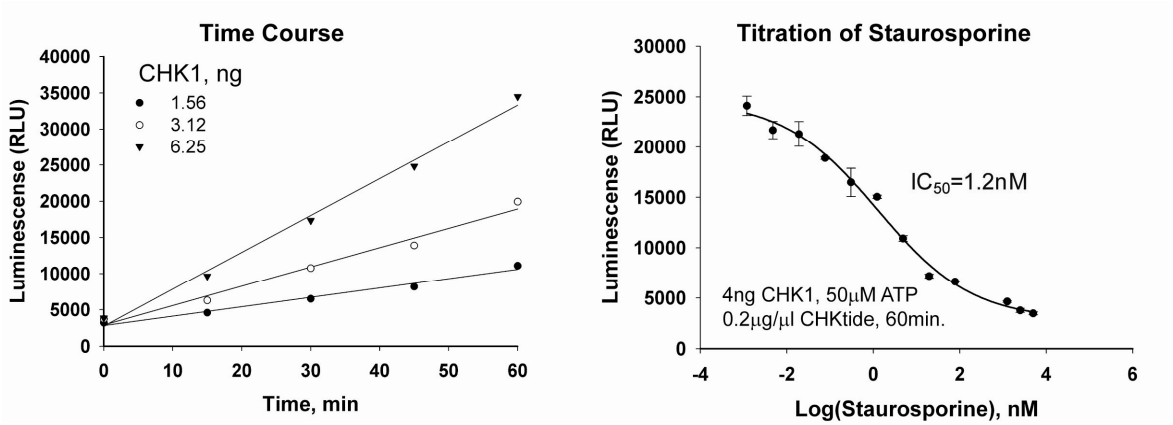

Figure 3. CHK1 Kinase Assay Development. CHK1 linear response curves were obtained at indicated amounts of enzyme using 0.2µg/µl of CHKtide peptide substrate and 50µM ATP. To determine the potency of the inhibitor (IC_{50}) staurosporine dose response was performed under conditions indicated in the figure.

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CHK1 Kinase Enzyme System	Promega	V1941
ADP-Glo + CHK1 Kinase Enzyme System	Promega	V9241

CHK1 Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
promega.com