ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CHK2

CHK2 Kinase Assay

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Scientific Background:

CHK2 is rapidly phosphorylated and activated in response to replication blocks and DNA damage; the response to DNA damage occurs in an ataxia telangiectasia mutated (ATM)-dependent manner (1). Expression of wild-type Chk2 leads to increased p53 stabilization after DNA damage, whereas expression of a dominant-negative Chk2 mutant abrogated both phosphorylation of p53 on Ser-20 and p53 stabilization (2).

1. Matsuoka, S. et al: Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science. 1998 Dec 4;282(5395):1893-7.

2. Chehab NH. et al: Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. Genes Dev. 2000 Feb 1;14(3):278-88.

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.

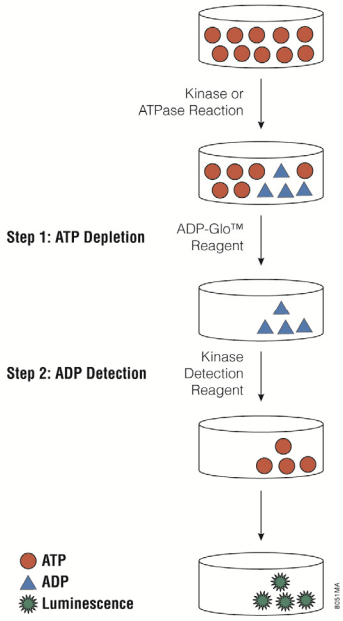

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

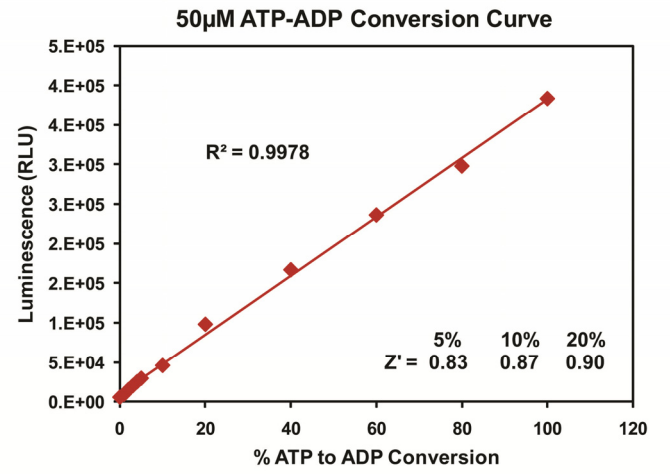

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. CHK2 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.

CHK2, ng	200	100	50	25	12.5	6.3	3.1	1.6	0
RLU	311881	262463	238758	169192	109325	41691	7830	3347	1806
S/B	173	145	132	94	61	23	4	1.9	1
% Conversion	92	77	70	50	32	12	2	0.9	0

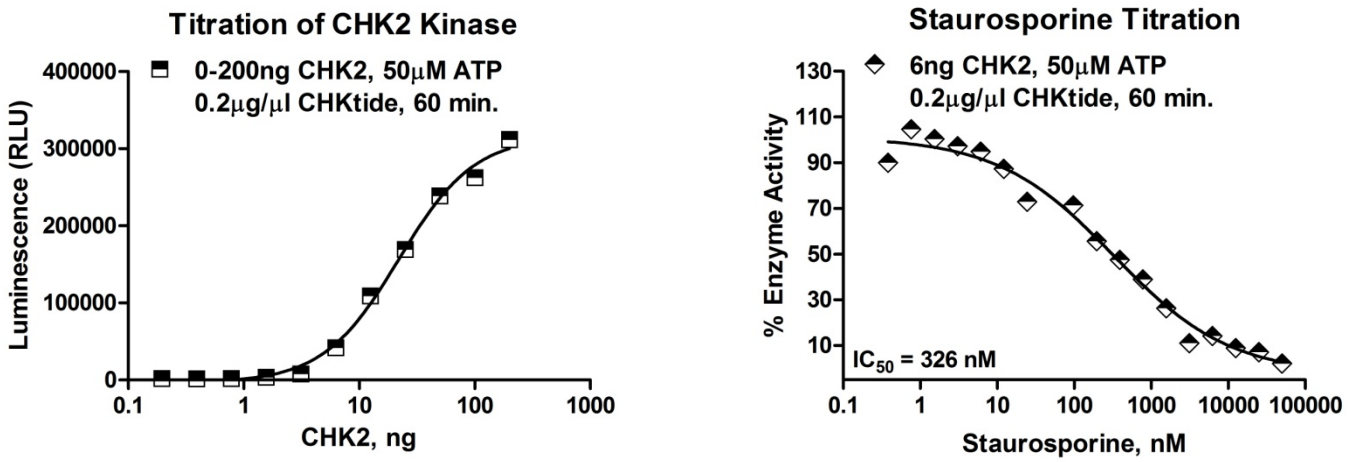

Figure 3. CHK2 Kinase Assay Development. (A) CHK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 6ng of CHK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CHK2 Kinase Enzyme System	Promega	V4020
ADP-Glo™ + CHK2 Kinase Enzyme System	Promega	V4021

CHK2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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