ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CK1α1

CK1α1 Kinase Assay

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Scientific Background:

CK1α1 is a member of the CK1 family of serine/threonine protein kinases which play an important role in diverse cell processes. CK1α1 can regulate Smo cell surface accumulation and activity in response to hedgehog. CK1α1 phosphorylate Smo at several sites and phosphorylation-deficient forms of Smo fail to accumulate on the cell surface and are unable to transduce the hedgehog signal (1). CK1α1 dynamically associates with the CBM complex on T cell receptor engagement to participate in cytokine production and lymphocyte proliferation. CK1α1 can form complex with MDM2 which then regulates the stability of p53 and E2F-1 transcription factors (2).

1. Jia, J. et al: Hedgehog signalling activity of Smoothened requires phosphorylation by protein kinase A and casein kinase I. Nature 432: 1045-1050, 2004.

2. Huart, A S. et al: CK1alpha plays a central role in mediating MDM2 control of p53 and E2F-1 protein stability. J Biol Chem. 2009 Nov 20;284(47):32384-94.

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.

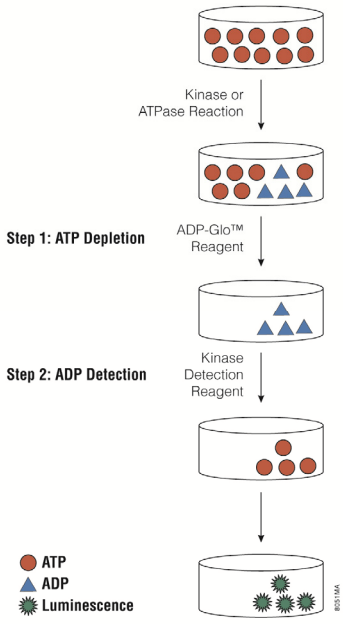

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

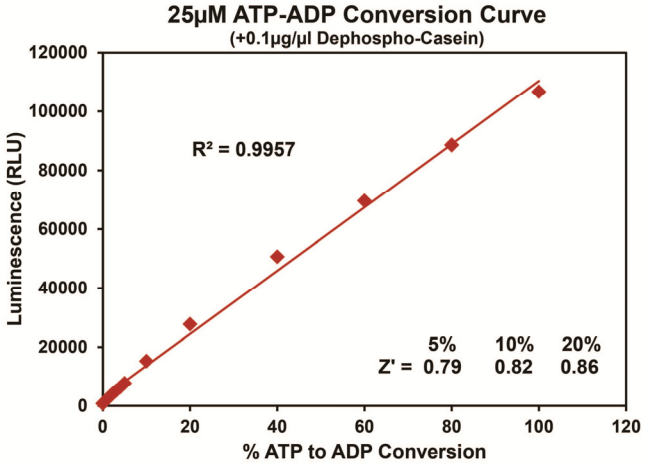

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. CK1α1 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.

CK1α1, ng	200	100	50	25	13	6.3	3.1	1.6	0
RLU	105579	45289	23436	11663	6985	3848	2380	1556	811
S/B	130	56	29	14	9	5	3	1.9	1
% Conversion	96	39	19	8	4	2	1	0.7	0

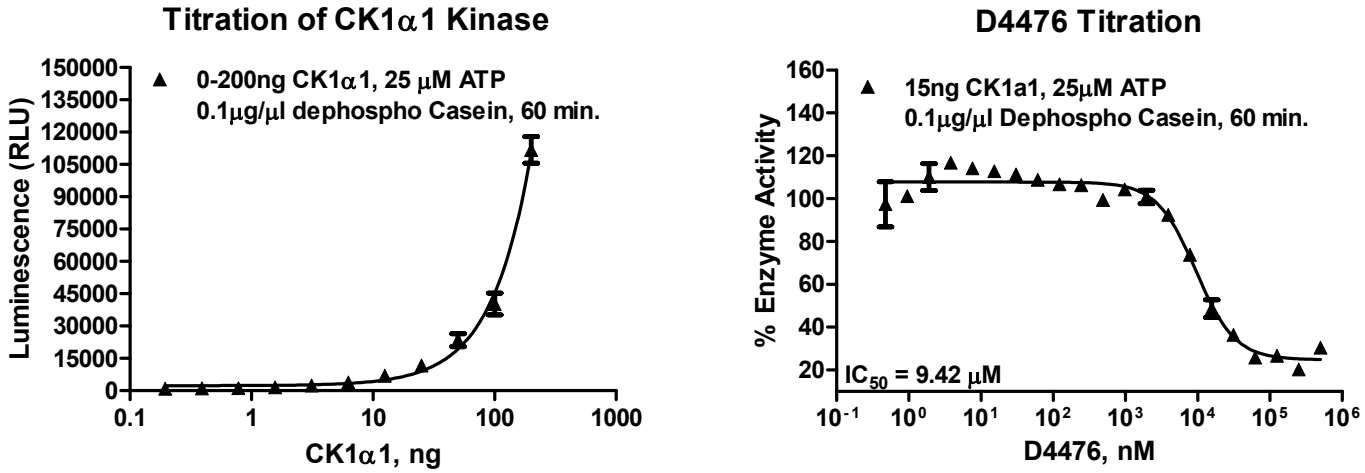

Figure 3. CK1α1 Kinase Assay Development. (A) CK1α1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) D4476 inhibitor dose response was created using 15ng of CK1α1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CK1α1 Kinase Enzyme System	Promega	V4484
ADP-Glo™ + CK1α1 Kinase Enzyme System	Promega	V4485

CK1α1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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