CK1ε Kinase Assay

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Scientific Background:

CK1ε is a member of the CK1 family of serine/threonine protein kinases which play an important role in diverse cell processes, including DNA replication and repair. CK1ε is a regulator of Yes-associated protein (YAP) transcription coactivator which is a key regulator of organ size and a candidate human oncogene. CK1ε is activated by CCK2R and this then phosphorylates PKD2 at Ser244. Phosphorylation of PKD2 leads to its nuclear accumulation and efficient phosphorylation of nuclear PKD2 substrates in human gastric cancer cells (1). CKIε can phosphorylate topoisomerase (topo) IIalpha at serine-1106 and this regulates the enzyme activity and sensitivity to topo II-targeted drugs (2).

1. von Blume J. et al: Phosphorylation at Ser244 by CK1 determines nuclear localization and substrate targeting of PKD2. EMBO J. 2007 Nov 14;26(22):4619-33.

2. Grozav, A G. et al: Casein kinase I delta/epsilon phosphorylates topoisomerase IIalpha at serine-1106 and modulates DNA cleavage activity. Nucleic Acids Res. 2009 Feb;37(2):382-92.

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.

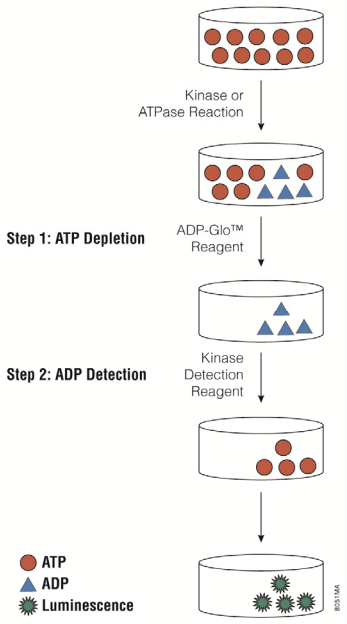

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

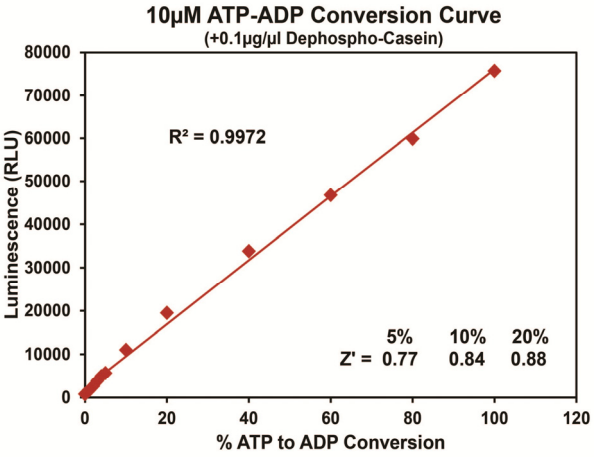

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. CK1ε 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.

CK1ε, ng	200	100	50	25	13	6.3	3.1	1.6	0
RLU	74983	59193	49802	36287	22090	12232	6087	3492	1462
S/B	51	40	34	25	15	8	4	2	1
% Conversion	98	77	64	46	27	14	5	2	0

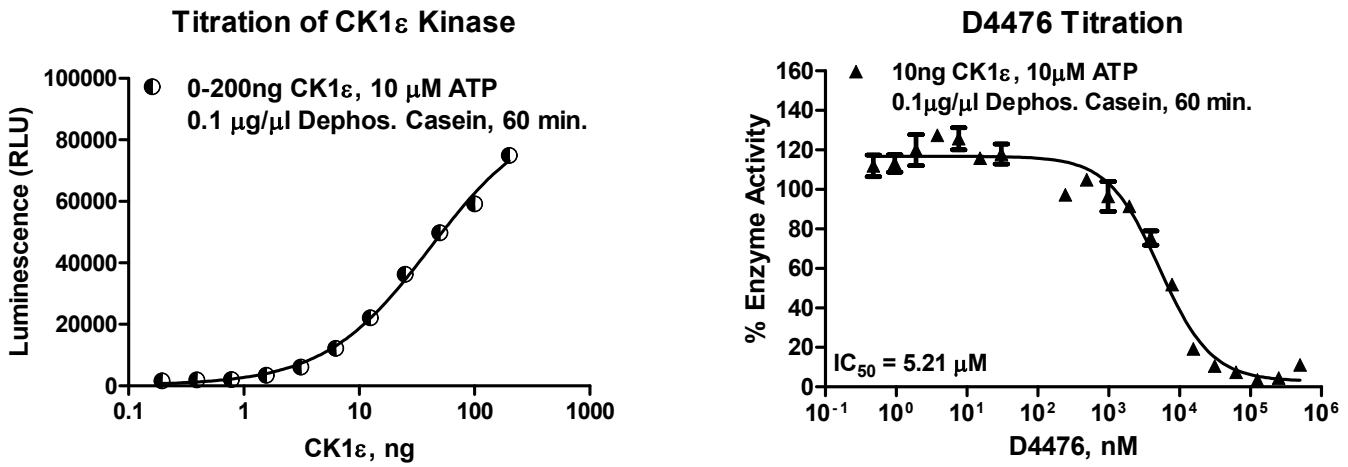

Figure 3. CK1ε Kinase Assay Development. (A) CK1ε enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) D4476 inhibitor dose response was created using 10ng of CK1ε to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CK1ε Kinase Enzyme System	Promega	V4160
ADP-Glo™ + CK1ε Kinase Enzyme System	Promega	V4161

CK1ε Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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