ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CK2α1

CK2α1 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:

CK2α is a serine-threonine protein kinase whose targets include many critical regulators of cellular growth. It is highly expressed in a lymphoproliferative disease of cattle and in many human cancers. Overexpression of the CK2 catalytic subunit in lymphocytes of transgenic mice leads to T cell lymphoma (1). The highest CK2α1 activity is found in mouse testicles and brain, followed by spleen, liver, lung, kidney and heart (2).

1. Rifkin, IR. et al: Acceleration of lymphoproliferative and autoimmune disease by transgenic protein kinase CK2 alpha. J Immunol. 1998 Nov 15;161(10):5164-70.

2. Guerra B. et al: Protein kinase CK2: evidence for a protein kinase CK2beta subunit fraction, devoid of the catalytic CK2alpha subunit, in mouse brain and testicles. FEBS Lett. 1999 Dec 3;462(3):353-7.

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.

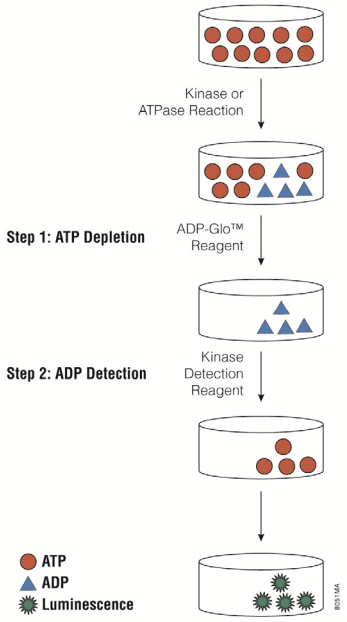

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

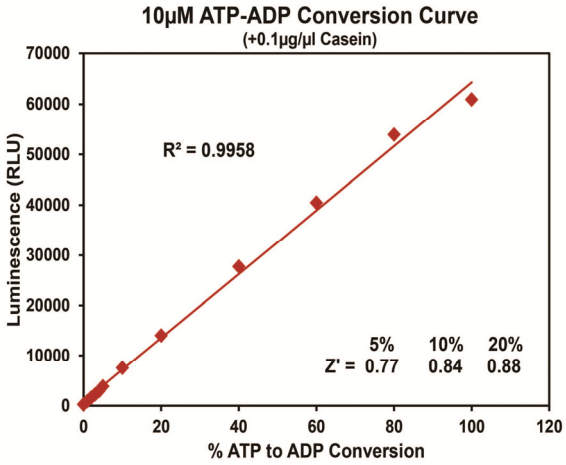

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. CK2α1 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.

CK2α1, ng	100	50	25	13	6	3.1	1.6	0.8	0
RLU	72373	40311	20475	8875	4137	2192	1242	802	303
S/B	239	133	68	29	14	7	4	3	1
% Conversion	100	58	29	12	6	3	1.4	0.8	0

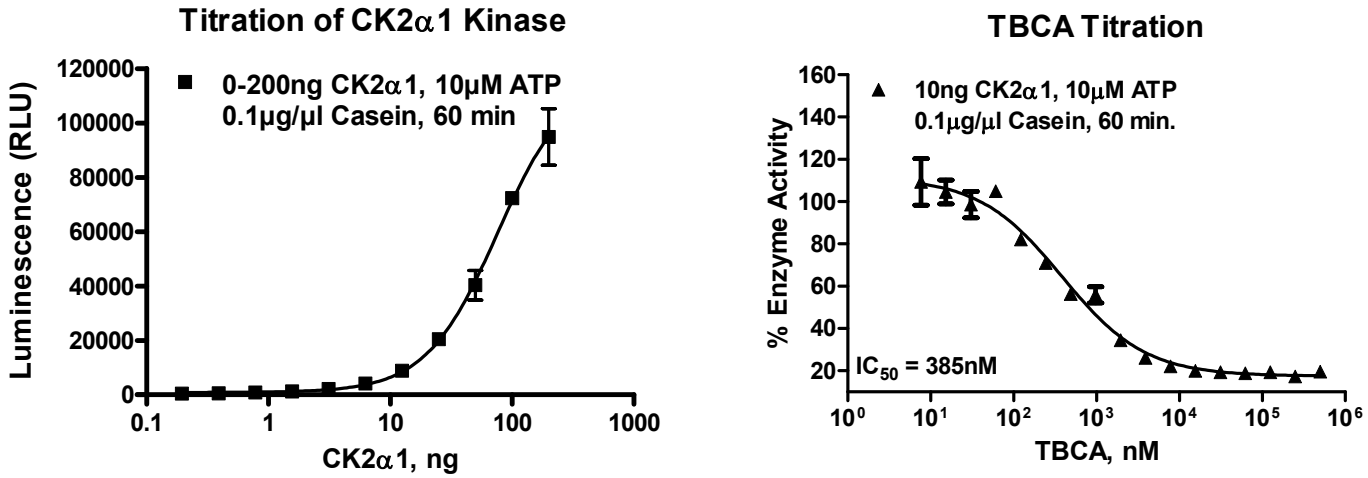

Figure 3. CK2α1 Kinase Assay Development. (A) CK2α1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) TBCA inhibitor dose response was created using 10ng of CK2α1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CK2α1 Kinase Enzyme System	Promega	V4482
ADP-Glo™ + CK2α1 Kinase Enzyme System	Promega	V4483

CK2α1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.