ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CAMKK1

CAMKK1 Kinase Assay

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Scientific Background:

CAMKK1 or CAMKKα is a Ca(2+)/calmodulin-dependent protein kinase that activates CaM-kinases I and IV via phosphorylation of their Thr(177) and Thr(196) residues, respectively. Recent studies have shown that the activity of CAMKK1 is decreased upon phosphorylation by cAMP-dependent protein kinase (PKA) (1) The CAMKKalpha has been identified in intact cells as AMPKKs, predicting a significant role for this kinase in regulating AMPK activity in vivo. It has been shown that 2-deoxyglucose- and ionomycin-stimulated AMPK activity is substantially reduced in HeLa cells transfected with small interfering RNAs specific for CAMKKα (2).

1. Okuno, S. et al: Regulation of Ca(2+)/calmodulin-dependent protein kinase kinase alpha by cAMP-dependent protein kinase: I. Biochemical analysis. J Biochem (Tokyo). 2001 Oct;130(4):503-13.

2. Hurley, R L. et al: The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. J Biol Chem. 2005 Aug 12;280(32):29060-6.

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.

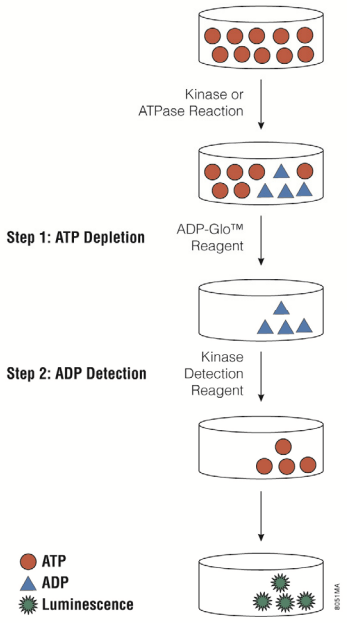

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

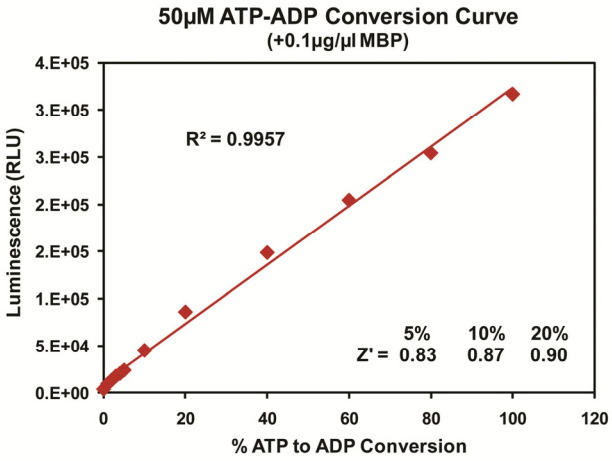

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. CAMKK1 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.

CAMKK1, ng	100	50	25	13	6	3.1	1.6	0
RLU	30087	15138	9562	4639	2945	2071	1630	1224
S/B	25	12	8	3.8	2.4	1.7	1.3	1
% Conversion	14	4	2	1	0.6	0.3	0.2	0

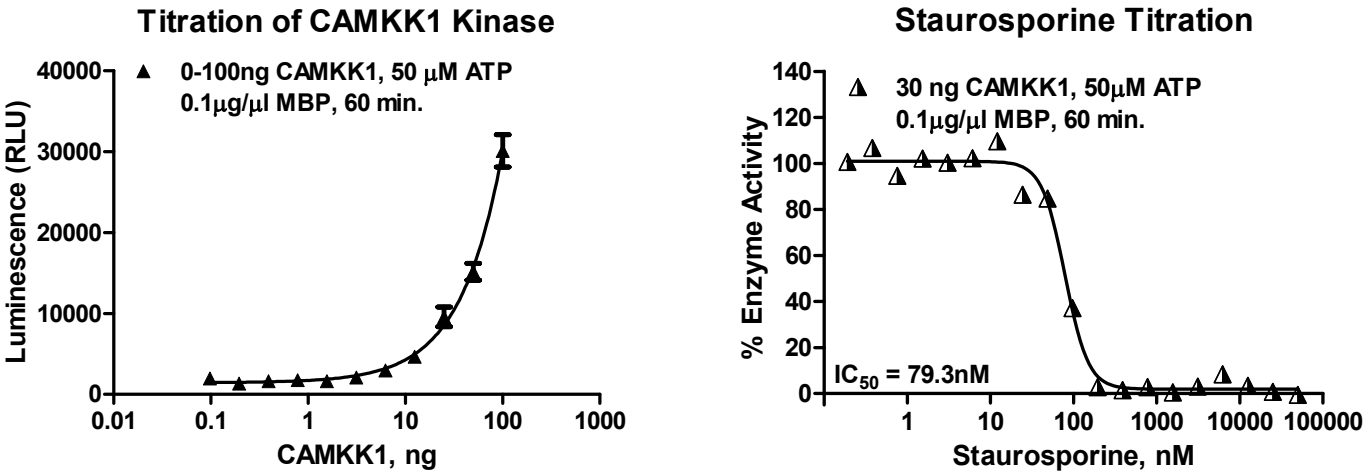

Figure 3. CAMKK1 Kinase Assay Development. (A) CAMKK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 30ng of CAMKK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CAMKK1 Kinase Enzyme System	Promega	V4470
ADP-Glo™ + CAMKK1 Kinase Enzyme System	Promega	V4471

CAMKK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT; Ca2+/Calmodulin solution (0.03µg/µl Calmodulin, 1mM Tris, pH 7.3, 0.5mM CaCl2)