ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CAMK2α

CAMK2α Kinase Assay

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Scientific Background:

CAMK2α is a ser/thr protein kinase that is a member of the Ca2+/calmodulin-dependent protein kinase family. CAMK2α is abundant in the brain as a major constituent of the postsynaptic density and is required for hippocampal long-term potentiation (LTP) and spatial learning. In addition to its Ca2+/calmodulin-dependent activity, CAMK2α can undergo autophosphorylation, resulting in Ca2+/calmodulin-independent activity. The protein level of CAMK2α fluctuates during neuronal activity in cultured rat pup hippocampal neurons. The levels of CAMK2α increased with heightened neuronal activity (2).

1. Silva, A J. et al: Impaired spatial learning in alpha-calcium calmodulin kinase II mutant mice. Science 257: 206-211, 1992.

2. Thiagarajan, T C. et al: Alpha- and beta-CaMKII: inverse regulation by neuronal activity and opposing effects on synaptic strength. Neuron 36: 1103-1114, 2002.

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.

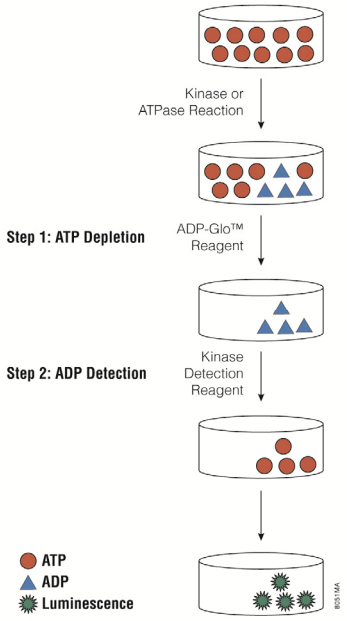

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

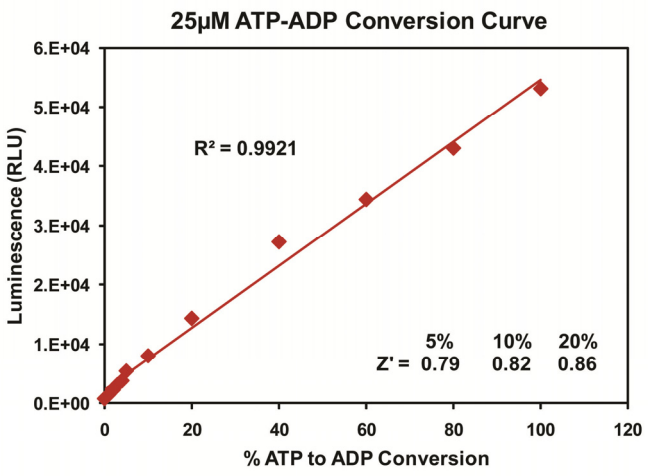

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. CAMK2α 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.

CAMK2α, ng	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0.1	0.05	0.02	0
RLU	54466	58502	46011	38305	24299	17157	12122	6393	2966	1921	418
S/B	130	140	110	92	58	41	29	15	7.1	4.6	1
% Conversion	41	44	34	29	18	13	9	5	2.1	1.3	0

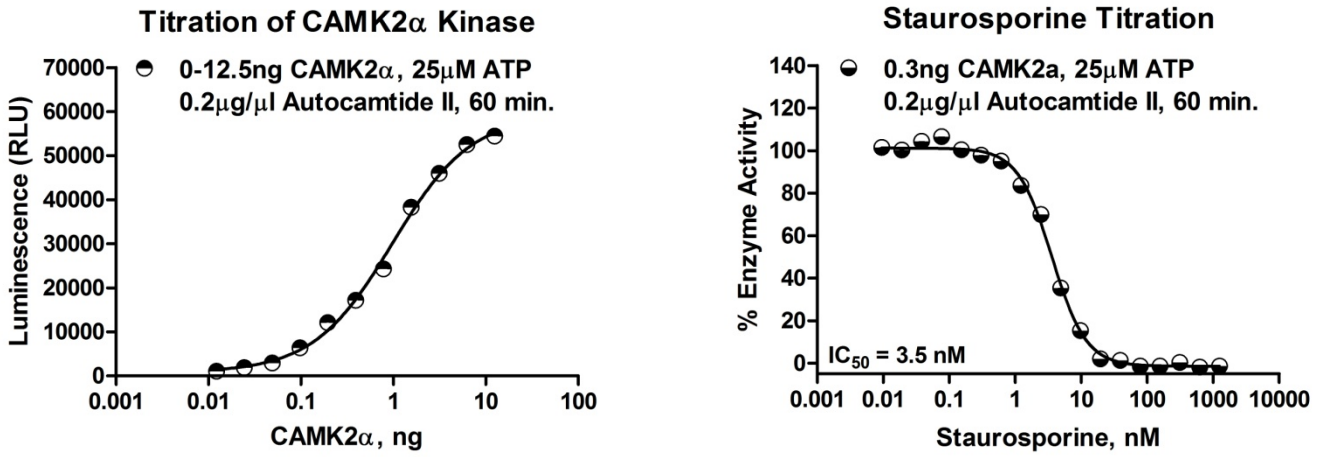

Figure 3. CAMK2α Kinase Assay Development. (A) CAMK2α enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.3ng of CAMK2α to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CAMK2α Kinase Enzyme System	Promega	V4018
ADP-Glo™ + CAMK2α Kinase Enzyme System	Promega	V4019

CAMK2α Kinase Buffer: 40mM Tris,pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT and Ca2+/Calmodulin solution (0.03µg/µl Calmodulin, 1mM Tris,pH 7.3 ,0.5mM $CaCl_2$).