ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

CAMK2β Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

CAMK2β belongs to the serine/threonine protein kinase family and to the type II multifunctional Ca(2+)/calmodulin-dependent protein kinase subfamily. CAMK2β showed wide tissue and cell distribution, and one of CAMK2β variant predominated in adult brain (1). The ratio of CAMK2α and CAMK2β protein levels were inversely related during activity in hippocampal neurons (2). CAMK2β is a prominent kinase in the central nervous system and may function in long-term potentiation and neurotransmitter release.

1. Tombes, R. M. et al: Identification of novel human tumor cell-specific CaMK-II variants. Biochim. Biophys. Acta 1355: 281-292, 1997.

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.

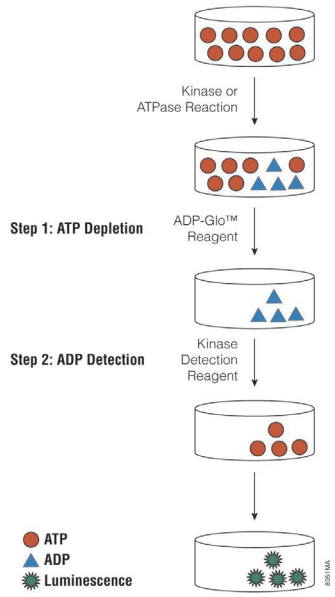

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

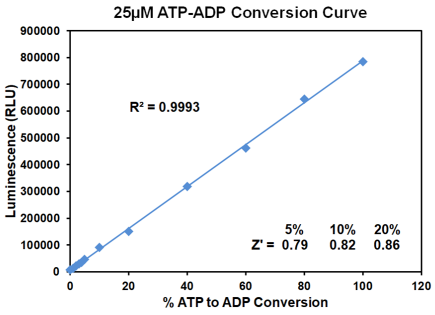

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	0
Luminescence	851,106	769,772	671,611	770,519	702,327	526,368	375,477	207,876	118,936	73,075	38,005	3,476
S/B	245	221	193	222	202	151	108	60	34	21	11	1
% Conversion	106	96	83	96	87	65	46	25	14	8	4	0

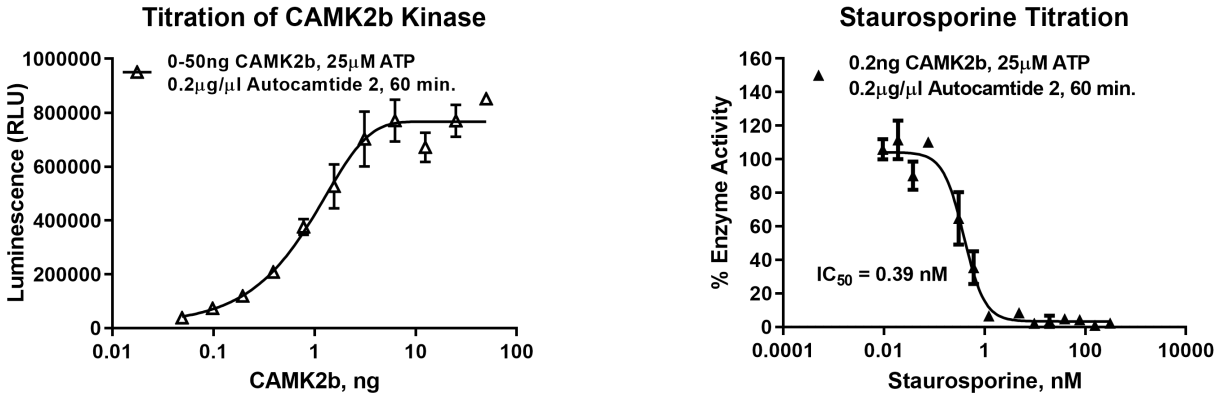

Figure 3. CAMK2β Kinase Assay Development. (A) CAMK2β enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 0.2ng of CAMK2β to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CAMK2β Kinase Enzyme System	10µg	VA7396
	1mg	VA7397
ADP-Glo™ + CAMK2β Kinase Enzyme System	1 Each	VA7398