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

CAMK2δ Kinase Assay

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Scientific Background:

CAMK2δ is a serine/threonine protein kinase that is a member of the type II multifunctional Ca2+/calmodulin-dependent protein kinase family. CAMK2δ is abundantly present in human cardiac and skeletal muscle and its levels are increased in the heart of patients suffering from cardiomyopathy (1). In cardiomyocytes, stimulation of beta-1-adrenergic receptor leads to induction of apoptosis, an effect that is mediated by activation of CAMK2δ in a PKA-independent manner. In addition, expression studies have revealed the downregulation of CAMK2δ in human tumor cells.

1. Hoch, B. Et al: Identification and expression of delta-isoforms of the multifunctional Ca(2+)/calmodulin-dependent protein kinase in failing and nonfailing human myocardium. Circ. Res. 84: 713-721, 1999.

2. Zhu, W.-Z. Et al: Linkage of beta-1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca(2+)/calmodulin kinase II. J. Clin. Invest. 111: 617-625, 2003.

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.

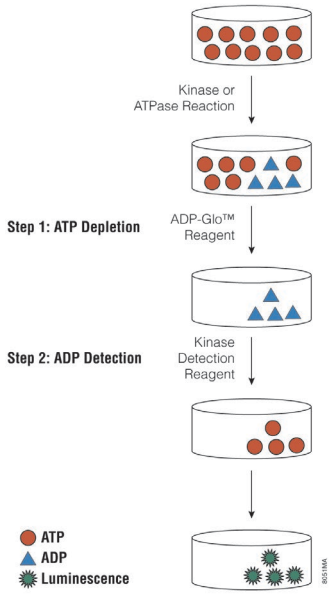

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

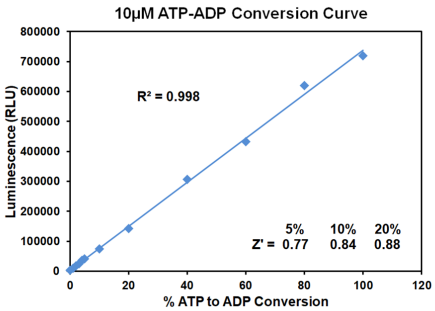

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	10	5	2.50	1.25	0.63	0.31	0.16	0.08	0.02	0.01	0
Luminescence	147,049	120,718	125,454	97,770	80,638	50,008	30,138	14,646	4,662	2,939	990
S/B	149	122	127	99	81	51	30	15	5	3	1
% Conversion	32	26	27	20	17	9	5	1	0	0	0

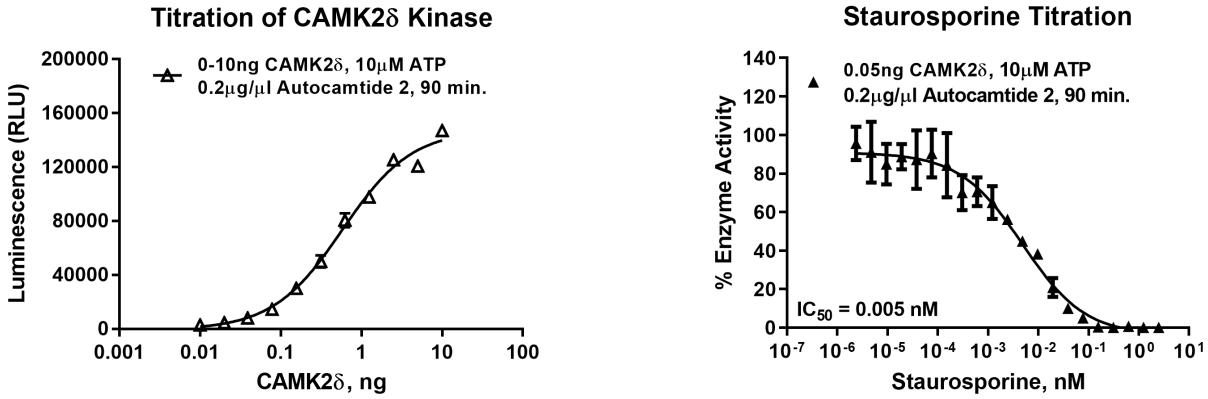

Figure 3. CAMK2δ Kinase Assay Development. (A) CAMK2δ enzyme was titrated using 10 µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 0.05 ng of CAMK2δ to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CAMK2δ Kinase Enzyme System	10µg	VA7036
	1mg	VA7037
ADP-Glo™ + CAMK2δ Kinase Enzyme System	1 Each	VA7038