

CAMK1 γ Kinase Assay

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Scientific Background:

CAMK1 γ (CLICK-III), a member of CAMK family, is a novel membrane-anchored neuronal Ca²⁺/calmodulin-dependent protein kinase. Full activation of CaMK1 γ requires both Ca²⁺/CaM and phosphorylation by CAMKK. CAMK1 γ transcripts is most abundant in neurons, with the highest levels in limited nuclei such as the central nucleus of the amygdala (CeA) and the ventromedial hypothalamus (1).

1. Takemoto-Kimura, S. et al: Molecular cloning and characterization of CLICK-III/CaMK1 γ , a novel membrane-anchored neuronal Ca²⁺/calmodulin-dependent protein kinase (CaMK). J. Biol. Chem. 278 (20), 18597-18605 (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.

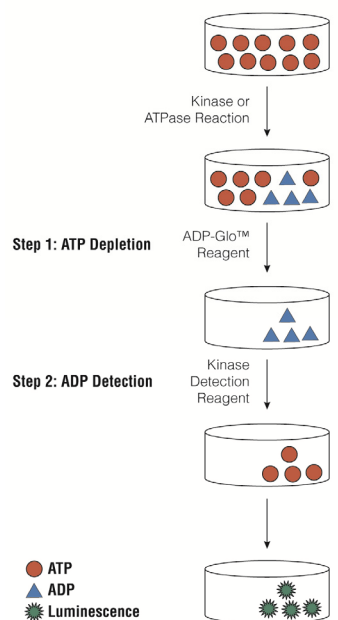


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

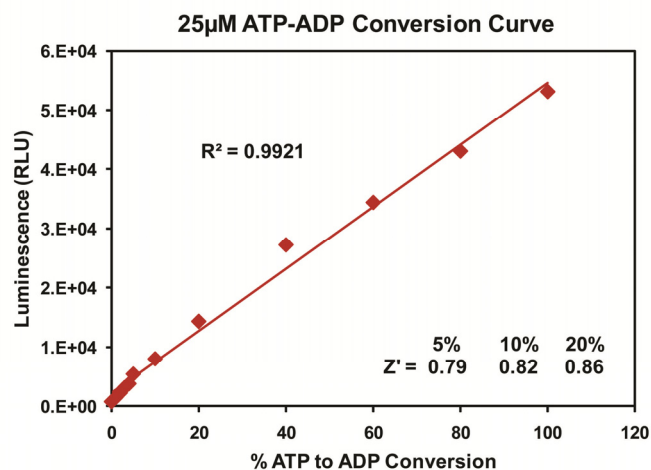
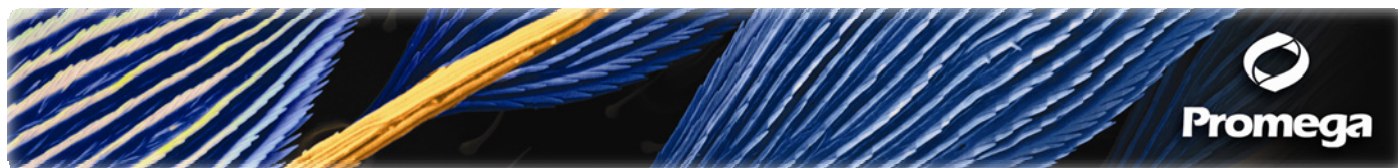


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25 μ M ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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 μ l of inhibitor or (5% DMSO)
 - 2 μ l of enzyme (defined from table 1)
 - 2 μ l of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 μ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. CAMK1 γ 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.

CAMK1 γ , ng	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	81405	70979	68892	50122	29375	16563	6783	3487	2041	1192	666
S/B	122	107	103	75	44	25	10	5.2	3.1	1.8	1
% Conversion	53	46	45	33	19	11	4	2.3	1.3	0.8	0

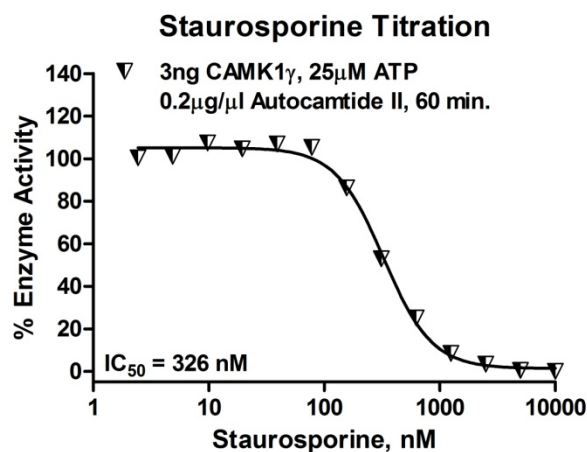
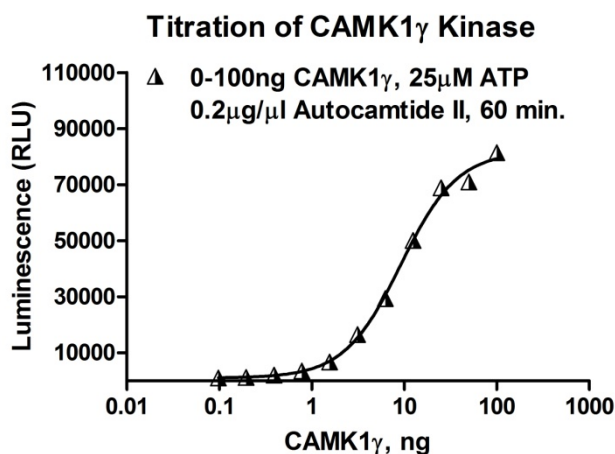


Figure 3. CAMK1 γ Kinase Assay Development. (A) CAMK1 γ enzyme was titrated using 25 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 3ng of CAMK1 γ to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:	Promega		SignalChem Specialists in Signaling Proteins
Products	Company	Cat.#	
ADP-Glo™ Kinase Assay	Promega	V9101	
CAMK1 γ Kinase Enzyme System	Promega	V4016	
ADP-Glo™ + CAMK1 γ Kinase Enzyme System	Promega	V4017	
CAMK1 γ Kinase Buffer: 40mM Tris,7.5; 20mM MgCl ₂ ; 0.1mg/ml BSA; 50 μ M DTT and Ca ²⁺ /Calmodulin solution (0.03 μ g/ μ l Calmodulin, 1mM Tris,pH 7.3 ,0.5mM CaCl ₂).			