ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CAMK2γ

CAMK2γ Kinase Assay

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Scientific Background:

CAMK2γ is a member of the CAMKII family which are ubiquitous serine/threonine protein kinases that have been implicated in diverse effects of hormones and neurotransmitters. CAMK2γ has six alternatively spliced variants that encode six different isoforms. Some of these variants have been identified in human tumors (1). Transgenic mice expressing a partially calcium-independent mutant form of CAMKG showed 1.5- to 2-fold increase in the thymus of these mice, at least in part due to an increase in the life span of double-positive thymocytes (2). There was an increase in the number of T cells in the secondary lymphoid organs that had acquired an antigen-dependent memory phenotype.

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

2. Bui, J. D. et al: A role for CaMKII in T cell memory. Cell 100: 457-467, 2000.

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.

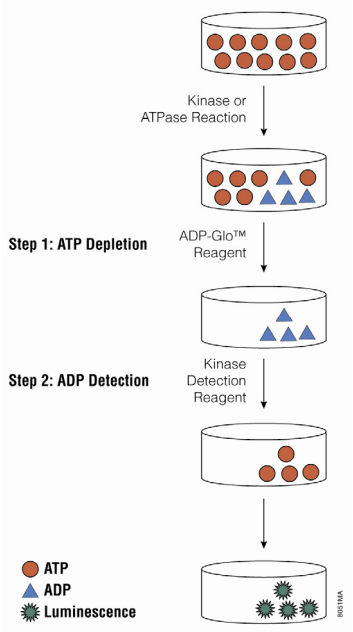

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

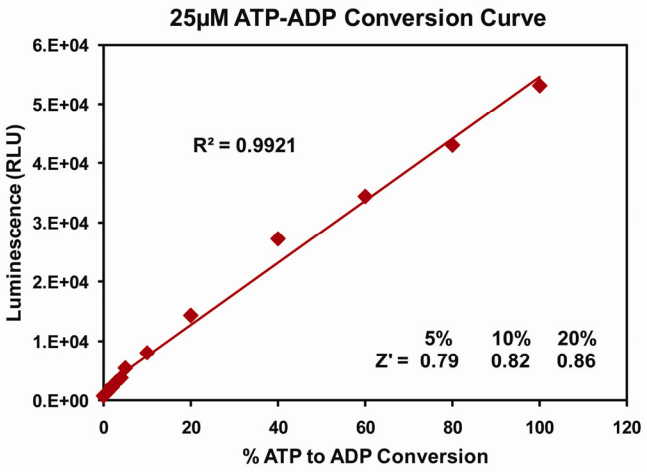

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 15 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. CAMK2γ 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.

CAMK2γ, ng	25.0	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0
RLU	33719	32788	40442	38539	28458	20139	15639	8284	4366	1021
S/B	33	32	37	33	28	20	15	8	4	1
% Conversion	100	100	100	100	86	60	45	21	9	0

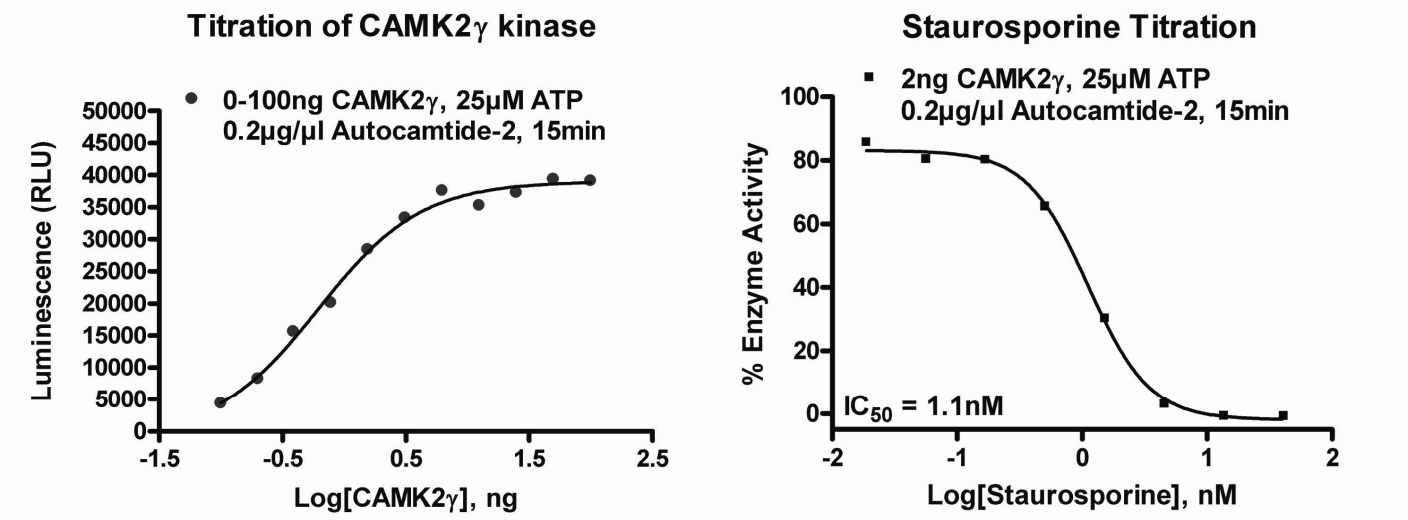

Figure 3. CAMK2γ Kinase Assay Development: (A) CAMK2γ enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of CAMK2γ to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CAMK2γ Kinase Enzyme System	Promega	V3531
ADP-Glo + CAMK2γ Kinase Enzyme System	Promega	V9201

CAMK2γ Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT; Ca2+/Calmodulin solution (0.03ug/ul Calmodulin, 1mM Tris, pH 7.3, 0.5mM $CaCl_2$).