ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: PKCα

PKCα Kinase Assay

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Scientific Background:

PKCα is a member of the protein kinase C (PKC) family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. PKC-alpha has been reported to play roles in many different cellular processes, such as cell adhesion, cell transformation, cell cycle checkpoint, and cell volume control(1). PKCα has been assigned to the chromosome region 17q22-q23.2 and has been identified as a fundamental regulator of cardiac contractility and Ca^{2+} handling in myocytes (2).

1. Coussens, L. et al: Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. Science 233: 859-866, 1986.

2. Braz, J C. et al: PKC-alpha regulates cardiac contractility and propensity toward heart failure. Nature Med. 10: 248-254, 2004.

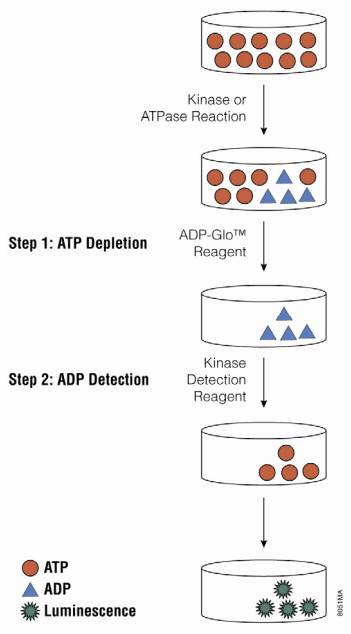

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

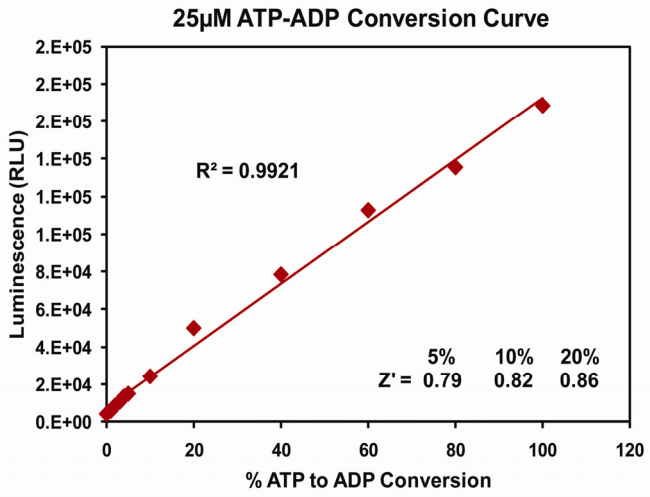

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 20 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. PKCα 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.

PKCα, ng	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0
RLU	153148	163360	160088	165687	159734	118372	90719	64860	36055	20455	3236
S/B	47	50	49	51	49	37	28	20	11	6	1
% Conversion	88	94	93	96	92	67	50	35	17	8	0

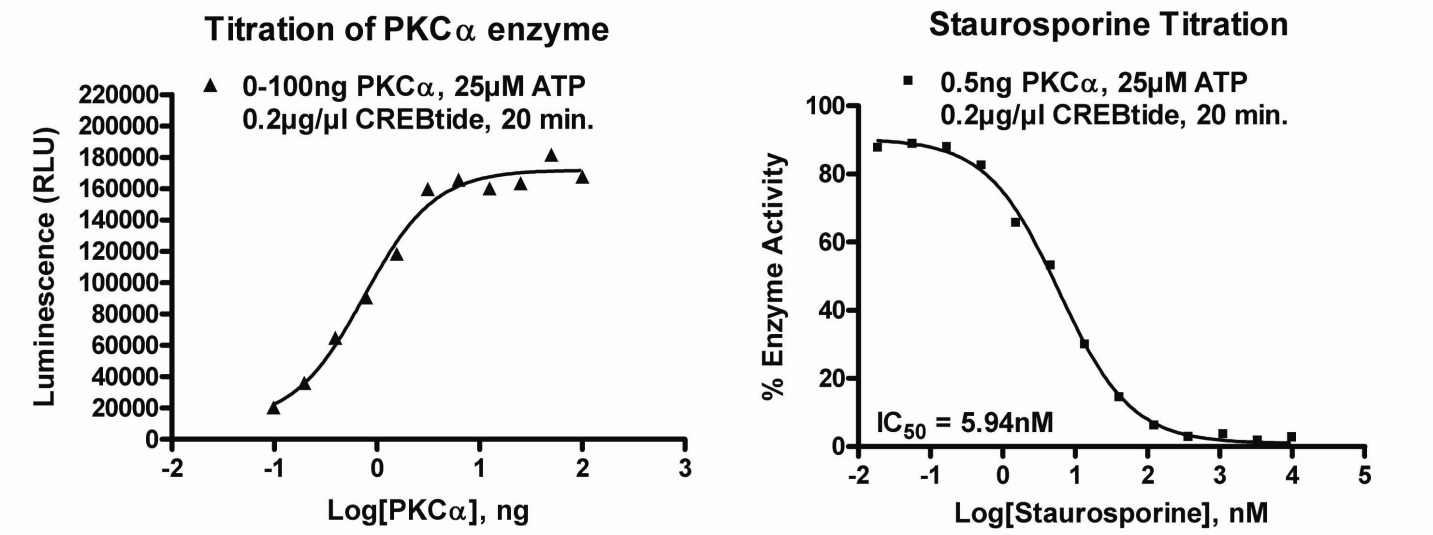

Figure 3. PKCα Kinase Assay Development: (A) PKCα enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.5ng of PKCα to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PKCα Kinase Enzyme System	Promega	V3381
ADP-Glo + PKCα Kinase Enzyme System	Promega	V9691

PKCα Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT; 1 x PKC Lipid activator mix.

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