PKCδ Kinase Assay

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Scientific Background:

Protein kinase C delta (PKCδ) is a member of the protein kinase C (PKC) family of serine-threonine kinases. It is a 79 kD protein kinase that shows strict dependence on the presence of phospholipids, but shows no activation by Ca2+ (1). Phosphatidylinositol is the most potent activator of PKC delta. Northern blot analysis indicated that PKCdelta is widely distributed in almost all the tissues and is a major isoform of PKC expressed in hemopoietic cells (2). PKCdelta is involved in fundamental cellular functions regulated by diacylglycerols and mimicked by phorbol esters.

1. Leibersperger, H. et al: Immunological demonstration of a calcium-unresponsive protein kinase C of the delta-type in different species and murine tissues. Predominance in epidermis. J Biol Chem. 1991 Aug 5;266(22):14778-84.

2. Mischak, H. et al: Mouse protein kinase C-delta, the major isoform expressed in mouse hemopoietic cells: sequence of the cDNA, expression patterns, and characterization of the protein. Biochemistry. 1991 Aug 13;30(32):7925-31.

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.

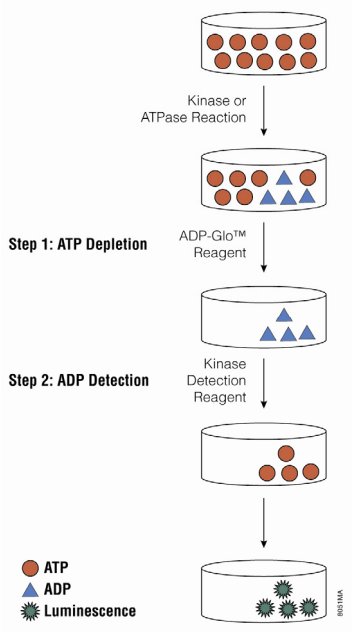

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

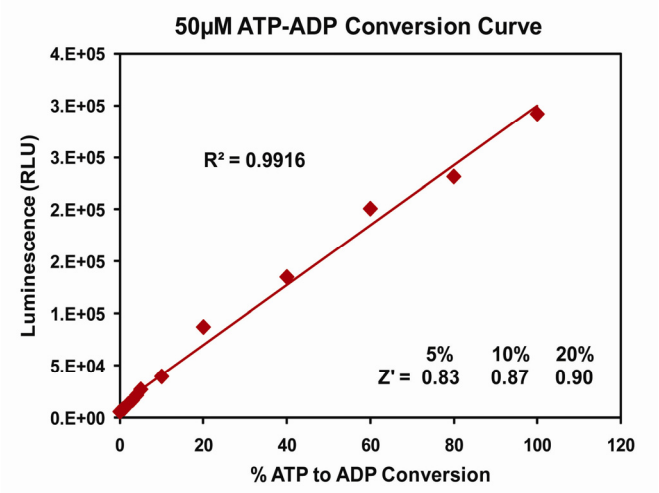

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. PKCδ 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.

PKCδ, ng	18.75	9.38	4.69	2.34	1.17	0.59	0.29	0
RLU	244149	257044	190643	117157	69432	41688	23097	4365
S/B	56	59	44	27	16	10	5	1
% Conversion	90	95	69	40	22	11	3	0

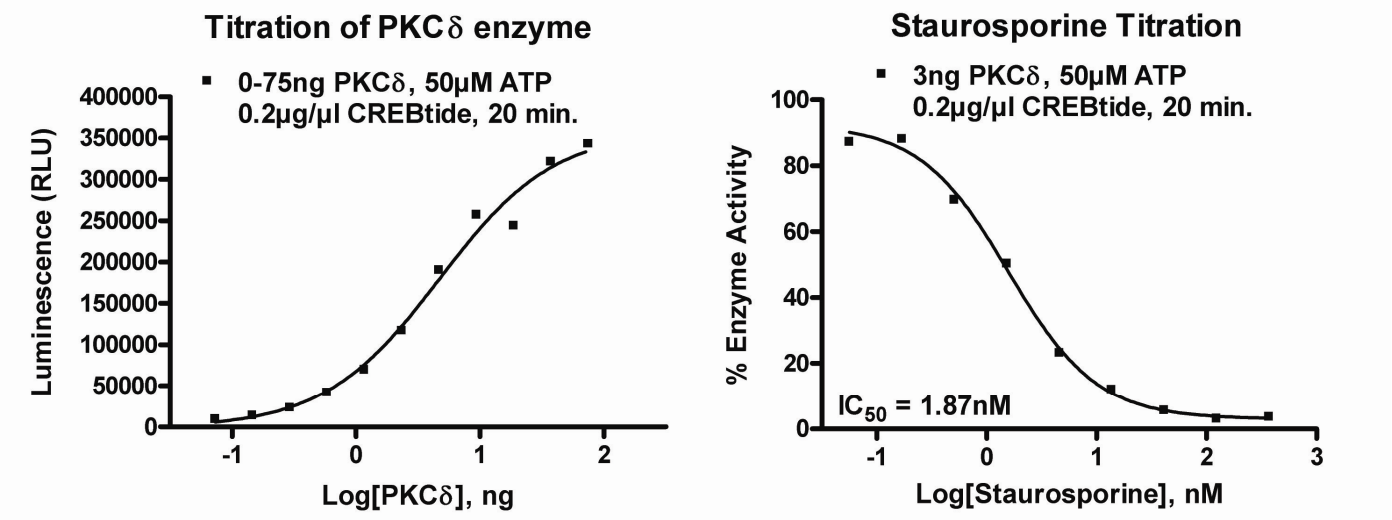

Figure 3. PKCδ Kinase Assay Development: (A) PKCδ enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 3ng of PKCδ to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PKCδ Kinase Enzyme System	Promega	V3401
ADP-Glo + PKCδ Kinase Enzyme System	Promega	V9721

PKCδ 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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