PKCμ (PKD1) Kinase Assay

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Scientific Background:

Protein kinase Cmu (PKCμ) is a member of the protein kinase C (PKC) family that differs from the other PKC isoenzymes in structural and enzymatic properties. PKCμ is a ubiquitous in nature with the highest expression in the thymus, lung and peripheral blood mononuclear cells (1). PKCμ forms a complex in vivo with a phosphatidylinositol 4-kinase and a phosphatidylinositol-4-phosphate 5-kinase. A region of PKCμ between the amino-terminal transmembrane domain and the pleckstrin homology domain is shown to be involved in the association with the lipid kinases (2).

1. Rennecke, J. et al: Immunological demonstration of protein kinase C mu in murine tissues and various cell lines. Eur J Biochem. 1996 Dec 1;242(2):428-32.

2. Nishikawa, K. et al: Association of protein kinase Cmu with type II phosphatidylinositol 4-kinase and type phosphatidylinositol-4-phosphate 5-kinase. J Biol Chem. 1998 Sep 4;273(36):23126-33.

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.

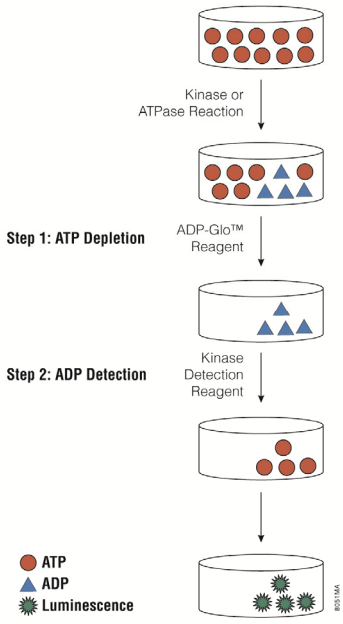

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

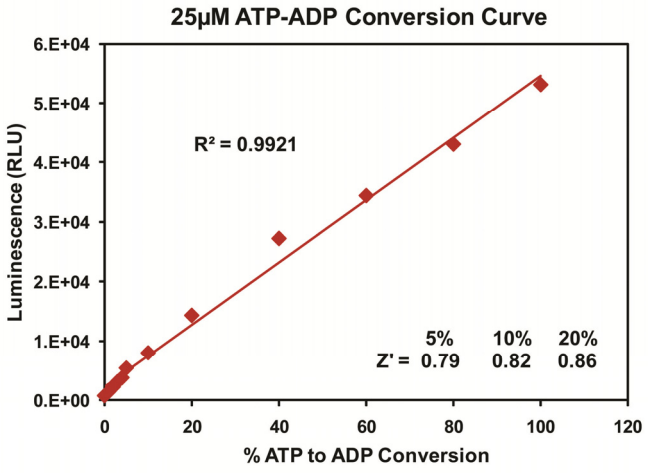

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 60 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. PKCµ 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.

PKCµ, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	127652	124370	113370	89433	66778	45809	27852	15782	8434	6214	506
S/B	252	246	224	177	132	91	55	31	17	12	1
% Conversion	99	97	88	69	52	35	21	12	6	5	0

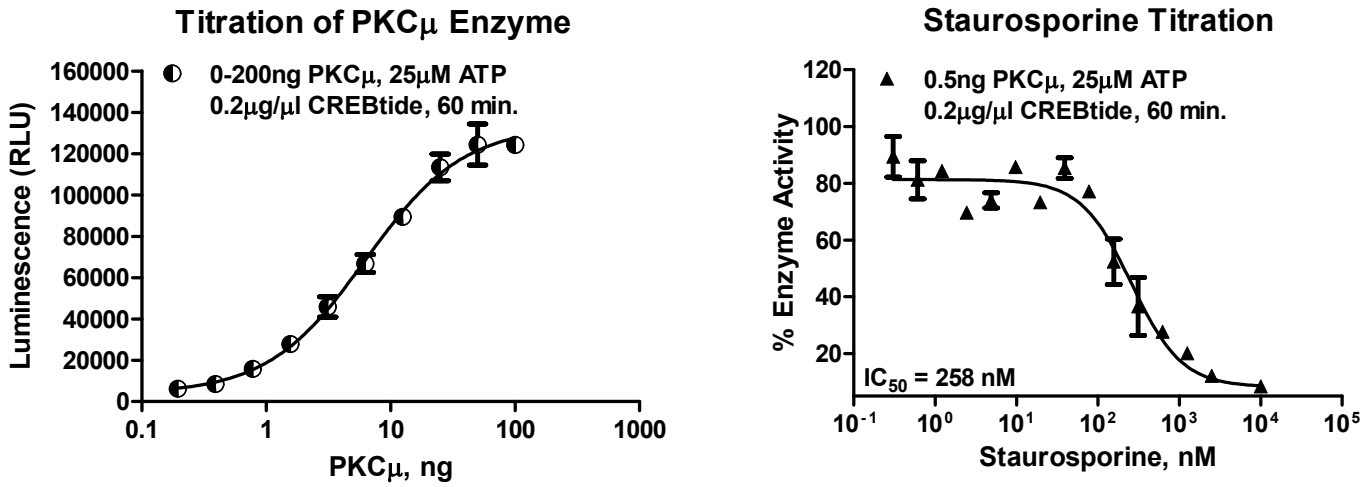

Figure 3. PKCµ Kinase Assay Development. (A) PKCµ enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.5ng of PKCµ to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PKCµ Kinase Enzyme System	Promega	V4038
ADP-Glo™ + PKCµ Kinase Enzyme System	Promega	V4039

PKCµ Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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