PKCι Kinase Assay

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Scientific Background:

PKCι is a member of the protein kinase C family of serine-threonine kinases. The amino acid sequence of PKCι showed greatest homology to PKCzeta, with 72% identity overall rising to 84% in the catalytic domain. Protein kinase C iota (PKCι) has been implicated in Ras signaling and is a critical downstream effector of oncogenic Ras in the colonic epithelium. Transgenic mice expressing constitutively active PKCι in the colon are highly susceptible to carcinogen-induced colon carcinogenesis (1).

1. Murray, N R. et al: Protein kinase C iota is required for Ras transformation and colon carcinogenesis in vivo. J Cell Biol. 2004 Mar 15;164(6):797-802.

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.

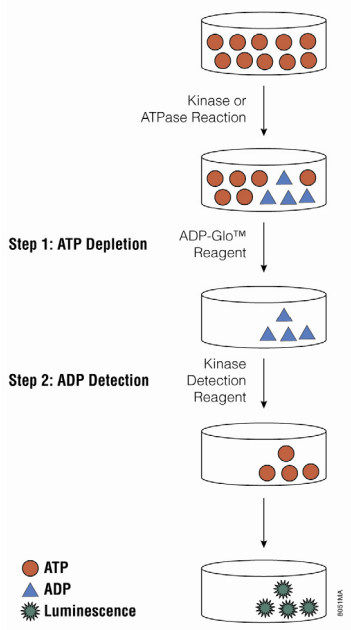


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

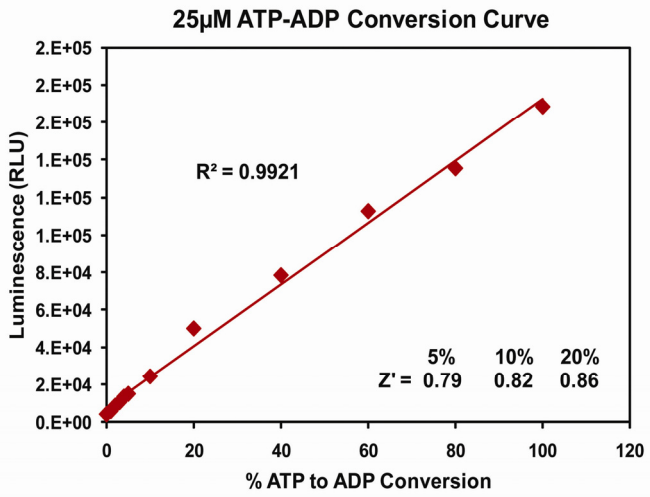


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

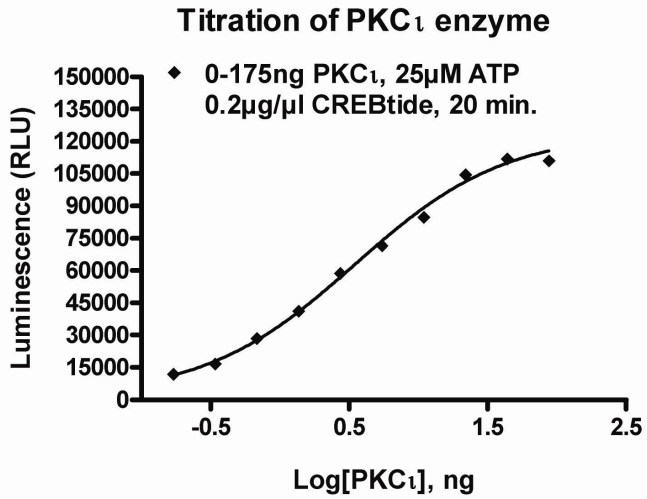
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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 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. PKCι 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.

PKCι, ng	175	88	44	21.9	10.9	5.5	2.7	1.4	0.7	0.3	0
RLU	190185	133155	111847	104469	77570	71472	58716	41124	28493	16600	2831
S/B	67	47	40	37	27	25	21	15	10	5.9	1
% Conversion	92	64	53	49	36	33	27	18	12	5.6	0



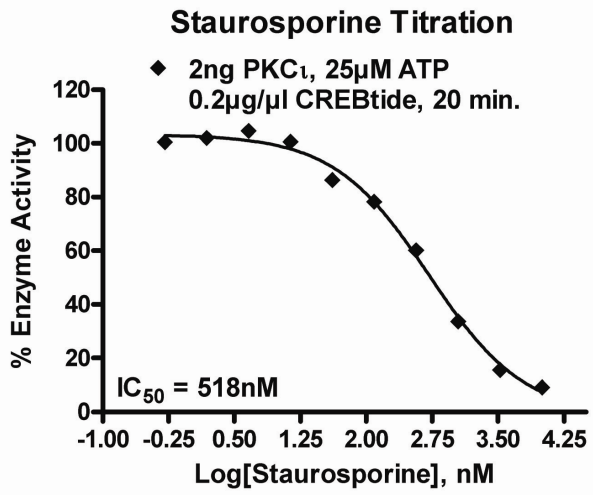


Figure 3. PKCι Kinase Assay Development: (A) PKCι enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of PKCι to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PKCι Kinase Enzyme System	Promega	V3751
ADP-Glo + PKCι Kinase Enzyme System	Promega	V9751

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