

## PKC $\zeta$ Kinase Assay

By Hicham Zegzouti, Ph.D., Jolanta Vidugiriene, Ph.D., and Said A. Goueli, Ph.D., Promega Corporation

### Scientific Background:

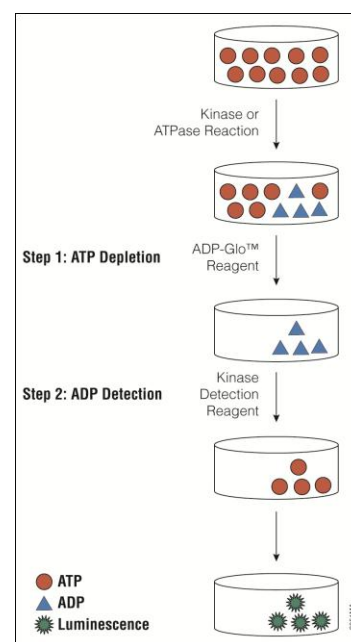
PKC $\zeta$  is an atypical isoform of the PKC family. PKC $\zeta$  is found in both particulate and soluble fractions and cannot be activated by phorbol ester. Overexpression of PKC $\zeta$  and subsequent phorbol ester treatment abolished phorbol ester-induced reduction in cell proliferation (1). Overexpression of PKC $\zeta$  also potentiates phorbol ester-induced mitogen-activated protein (MAP) kinase activation in a PKC-dependent manner. PKC $\zeta$  is an upstream modulator of p70S6K, an important regulator of cell proliferation (2).

1. Kim, SJ. Et al: Phorbol ester effects in atypical protein kinase C zeta overexpressing NIH3T3 cells: possible evidence for crosstalk between protein kinase C isoforms. *Biochem Biophys Res Commun.* 1997 Aug 18;237(2):336-9.
2. Romanelli, A. et al: p70 S6 kinase is regulated by protein kinase Czeta and participates in a phosphoinositide 3-kinase-regulated signalling complex. *Mol Cell Biol.* 1999 Apr;19(4):2921-8.

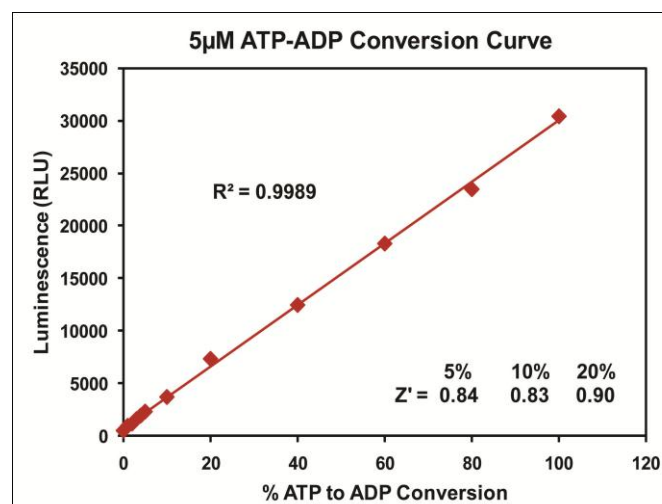
### ADP-Glo™ Kinase Assay

#### 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 5 $\mu$ M 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.



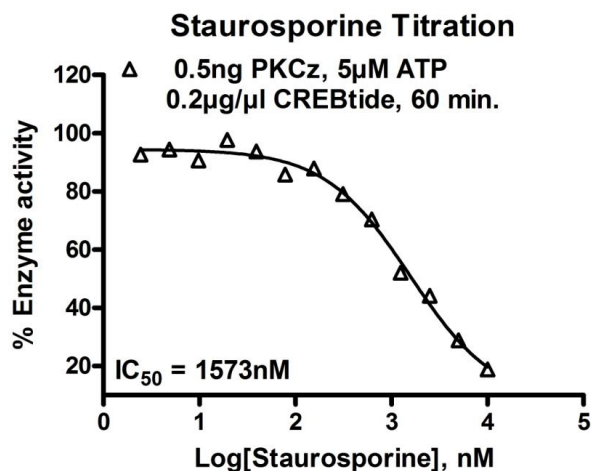
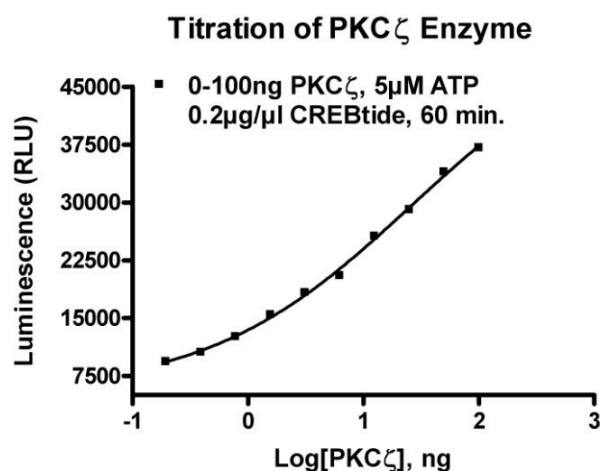
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html)

## Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1  $\mu$ l of inhibitor or (5% DMSO)
  - 2  $\mu$ l of enzyme (defined from table 1)
  - 2  $\mu$ l of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5  $\mu$ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10  $\mu$ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

**Table 1. PKC $\zeta$  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.

PKC $\zeta$ , ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	37076	33935	29048	25603	20506	18302	15447	12582	10558	9349	757
S/B	49	45	38	34	27	24	20	17	14	12	1
% Conversion	85	78	67	59	47	41	35	28	23	20	0



**Figure 3. PKC $\zeta$  Kinase Assay Development:** (A) PKC $\zeta$  enzyme was titrated using 5 $\mu$ M 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 PKC $\zeta$  to determine the potency of the inhibitor (IC<sub>50</sub>).

### Assay Components and Ordering Information:



#### Products

ADP-Glo™ Kinase Assay  
 PKC $\zeta$  Kinase Enzyme System  
 ADP-Glo + PKC $\zeta$  Kinase Enzyme System

#### Company

Promega  
 Promega  
 Promega

#### Cat.#

V9101  
 V2781  
 V9731

PKC $\zeta$  Kinase Buffer: 40mM Tris, 7.5; 20mM MgCl<sub>2</sub>; 0.1mg/ml BSA; 50 $\mu$ M DTT.