PKCβ1 Kinase Assay

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Scientific Background:

PKCβI is a member of the PKC family (phospholipid-dependent serine/threonine kinase) and is highly related to PKCβ II. Unlike the mature PKCβ II mRNA and protein, which rapidly increase following acute insulin treatment, the PKCβI mRNA and protein levels remain unchanged (1). The stable overexpression of PKCbetaII, but not PKCβI, leads to insulin-stimulated glucose uptake into cells. Upon stimulation of B lymphocytes and mast cells, Syk regulates Btk, and Btk selectively regulates enzymatic activity of PKCβI. Specific regulation of PKCβI by Btk is consistent with the selective association of Btk with PKCβI (2).

1. Cooper, D R. et al: Ectopic expression of protein kinase CbetaII, -delta, and -epsilon, but not -betaI or -zeta, provide for insulin stimulation of glucose uptake in NIH-3T3 cells. Arch Biochem Biophys. 1999 Dec 1;372(1):69-79.

2. Kawakami, Y. et al: Regulation of protein kinase CbetaI by two protein-tyrosine kinases, Btk and Syk. Proc Natl Acad Sci U S A. 2000 Jun 20;97(13):7423-8.

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.

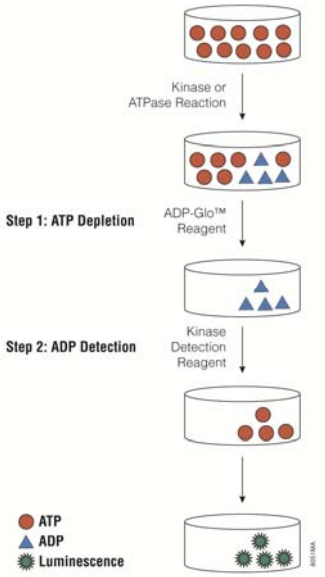


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

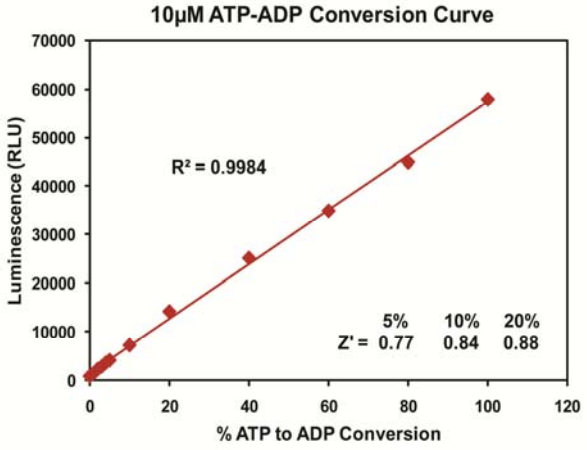


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

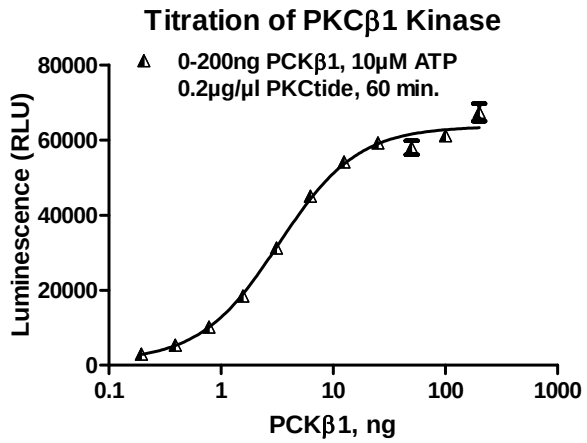
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

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 0.5-1sec).

Table 1. PKCβ1 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.

PKCβ1, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
Luminescence	67357	61137	57987	59237	54193	45005	31251	18435	10163	5376	2956	192
S/B	351	318	302	309	282	234	163	96	53	28	15	1
% Conversion	66	61	58	59	55	47	36	19	10	5	3	0



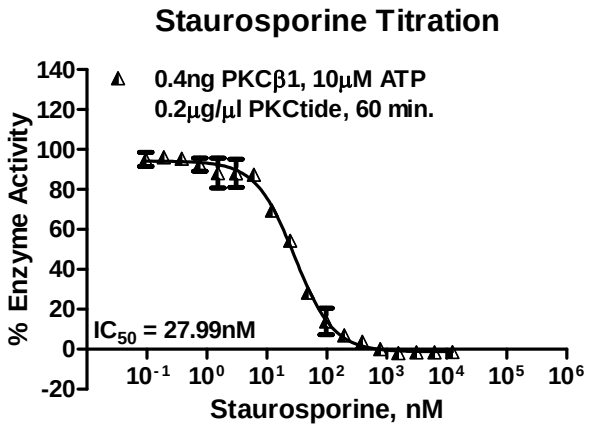


Figure 3. PKCβ1 Kinase Assay Development. **(A) PKCβ1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.4ng of PKCβ1 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PKCβ1 Kinase Enzyme System	Promega	V5094
ADP-Glo™ + PKCβ1 Kinase Enzyme System	Promega	V5095

PKCβ1 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT; 1 x PKC Lipid Activator mix.