ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: PKCθ

PKCθ Kinase Assay

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Scientific Background:

Protein Kinase C, theta (PKCθ) is important component in the intracellular signaling cascade (1). Recent studies have suggested that local accumulation of fat metabolites inside skeletal muscle may activate a serine kinase cascade involving PKC-theta leading to defects in insulin signaling and glucose transport in skeletal muscle (2). Insulin resistance plays a primary role in the development of type 2 diabetes and may be related to alterations in fat metabolism. PKC-theta is a crucial component mediating fat-induced insulin resistance in skeletal muscle and is a potential therapeutic target for the treatment of type 2 diabetes (2).

1. Manicassamy, S. and Sun, Z. The critical role of protein kinase C-theta in Fas/Fas ligand-mediated apoptosis. J. Immunol. 2007;178(1):312-9.

2. Kim, J K. et al: PKC-theta knockout mice are protected from fat-induced insulin resistance. J. Clin. Invest. 2004;114(6):823-7.

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.

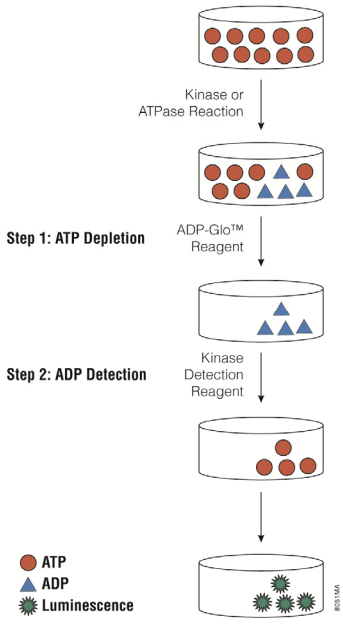

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

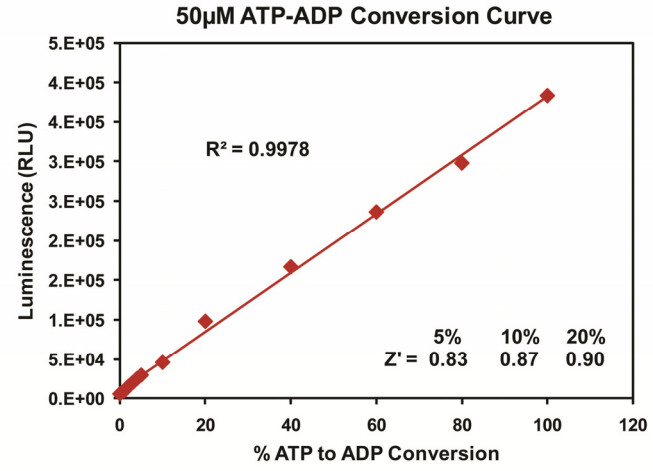

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. PKCθ 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.

PKCθ, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	228314	211205	195201	147664	100179	41791	20510	12067	5095	4408	1746
S/B	131	121	112	85	57	24	12	7	2.9	2.5	1
% Conversion	95	88	81	61	41	17	8	5	1.6	1.3	0

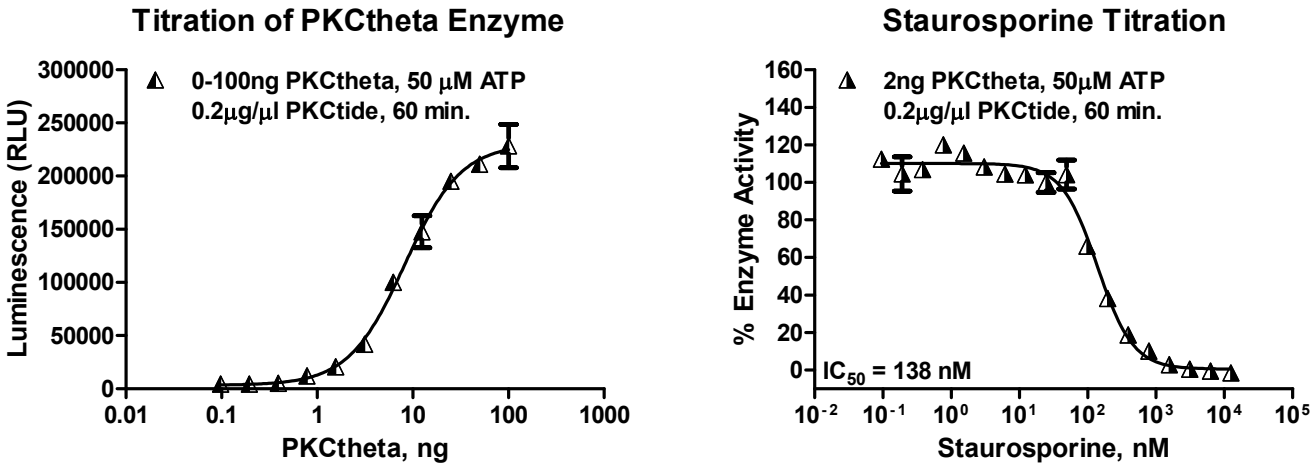

Figure 3. PKCθ Kinase Assay Development. **(A) PKCθ enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of PKCθ to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PKCθ Kinase Enzyme System	Promega	V4040
ADP-Glo™ + PKCθ Kinase Enzyme System	Promega	V4041

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