ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

PKC eta Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

PKCη is a member of the protein kinase C (PKC) family of serine- and threonine-specific protein kinases that can phosphorylate a wide variety of protein targets known to be involved in diverse cellular signaling pathways. PKCη is predominantly expressed in squamous cell epithelia and induces terminal differentiation of keratinocytes. PKCη that is endogenously expressed or overexpressed is found to associate with the cyclin E/cdk2/p21 complex in keratinocytes of mice and humans (1).

1. Kashiwagi, M. et al: PKCη associates with cyclin E/cdk2/p21 complex, phosphorylates p21 and inhibits cdk2 kinase in keratinocytes. Oncogene. 2000 Dec 14;19(54):6334-41.

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.

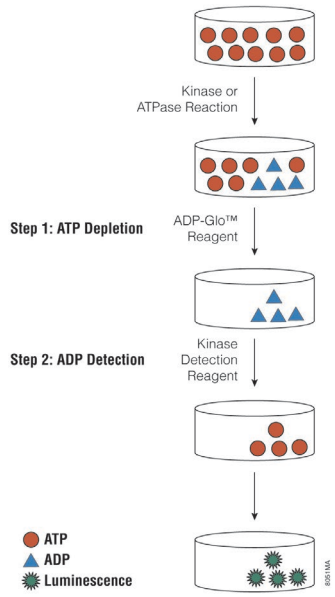

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

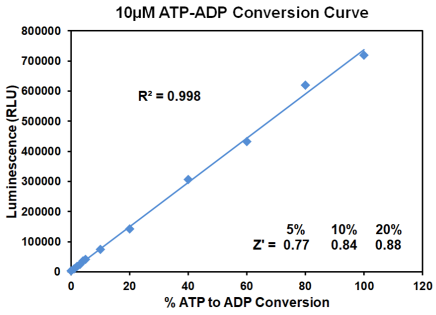

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0
Luminescence	216,172	209,918	202,612	173,730	149,940	91,812	50,614	25,127	13,015	5,942	1,240
S/B	174	169	163	140	121	74	41	20	10	5	1
% Conversion	47	46	44	38	33	19	10	4	2	0	0

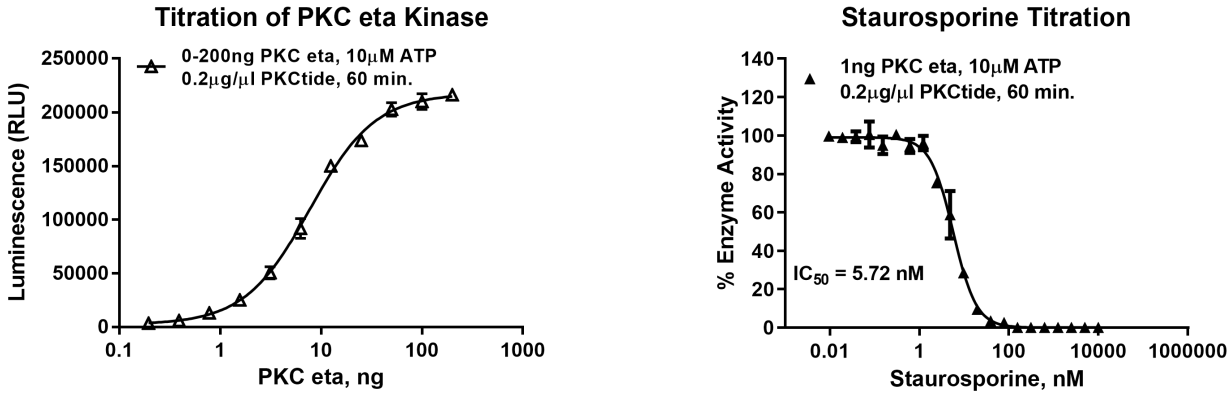

Figure 3. PKC eta Kinase Assay Development. (A) PKC eta enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of PKC eta to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PKC eta Kinase Enzyme System	10µg	VA7531
	1mg	VA7532
ADP-Glo™ + PKC eta Kinase Enzyme System	1 Each	VA7533