ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

PKC nu Kinase Assay

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Scientific Background:

PKCv, also known as PKD3, is a member of the protein kinase C (PKC) family of serine/threonine kinases that play critical roles in the regulation of cellular differentiation and proliferation in many cell types. PKCv is composed of 890 amino acid residues and the protein has 77.3% similarity to human PKC mu (PKCμ) and 77. 4% similarity to mouse PKD (1). The PKCv mRNA is ubiquitously expressed in various tissues and the gene is located between markers WI-9798 and D2S177 on chromosome 2p21 region.

1. Hayashi,A. et al: PKCnu, a new member of the protein kinase C family, composes a fourth subfamily with PKCmu. Biochim Biophys Acta. 1999 May 6;1450(1):99-106.

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.

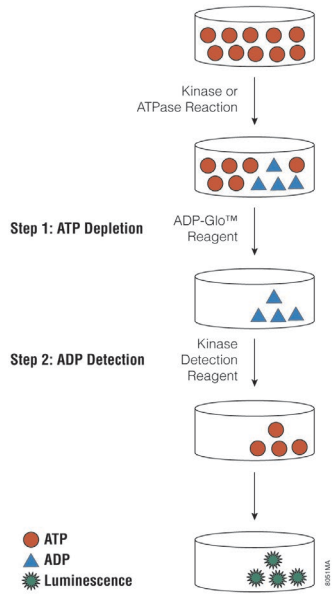

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

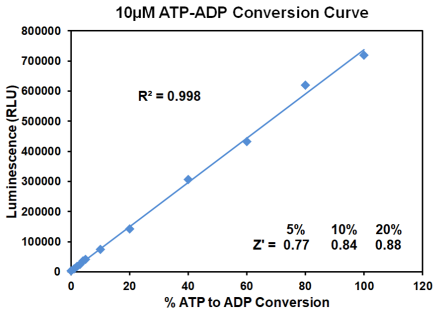

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	120	60	30	15	7.50	3.75	1.88	0.94	0.47	0
Luminescence	230,422	201,552	152,117	94,420	53,058	27,873	18,033	10,995	8,045	4,742
S/B	49	42	32	20	11	6	4	2	2	1
% Conversion	53	46	35	22	12	6	4	2	2	0

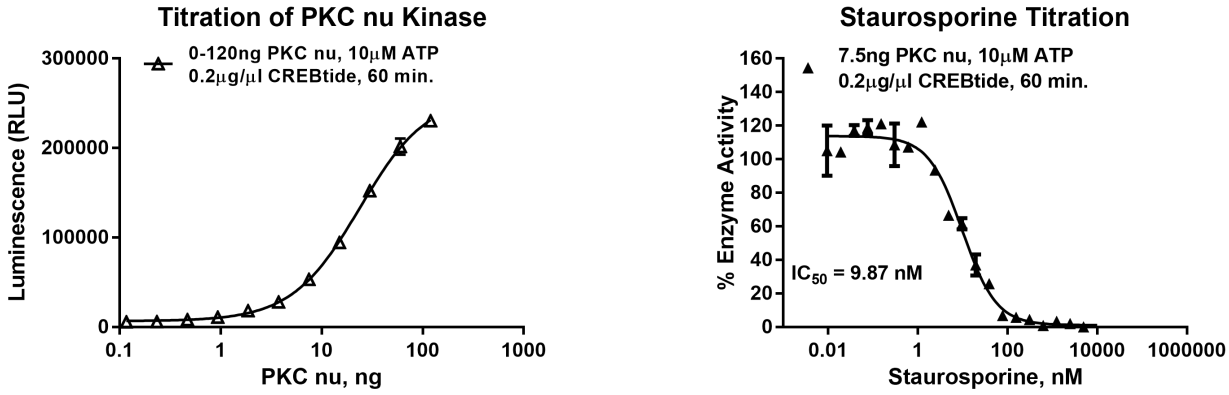

Figure 3. PKC nu Kinase Assay Development. (A) PKC nu enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 7.5ng of PKC nu to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PKC nu Kinase Enzyme System	10µg	VA7534
	1mg	VA7535
ADP-Glo™ + PKC nu Kinase Enzyme System	1 Each	VA7536