ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

PRKX Kinase Assay

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Scientific Background:

PRKX is a serine/threonine protein kinase that is closely related to the catalytic subunit of the cAMP-dependent protein kinase. PRKX regulates epithelial morphogenesis during mammalian kidney development and PRKX expression markedly activates migration of cultured renal epithelial cells in the presence of cAMP. Aberrant adult kidney expression of PRKX is found in autosomal dominant polycystic kidney disease (1). PRKX is involved in macrophage and granulocyte maturation. Abnormal recombination between PRKX and a related pseudogene on chromosome Y is a frequent cause of sex reversal disorder in XX males and XY females (2).

1. Li, X. et al: PRKX, a phylogenetically and functionally distinct cAMP-dependent protein kinase, activates renal epithelial cell migration and morphogenesis. Proc. Nat. Acad. Sci. vol. 99, 9260-9265, 2002.

2. Klink, A. et al: The human protein kinase gene PKX1 on Xp22.3 displays Xp/Yp homology and is a site of chromosomal instability. Hum. Molec. Genet. vol. 4, 869-878, 1995.

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.

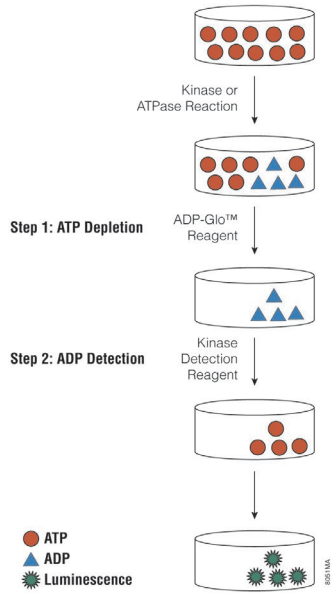

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

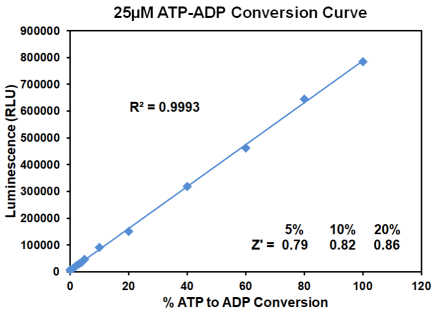

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0.29	0
Luminescence	523,980	434,220	325,619	233,952	137,867	71,556	39,033	21,204	11,251	7,761	3,316
S/B	158	131	98	71	42	22	12	6	3	2	1
% Conversion	61	51	38	27	15	7	3	1	0	0	0

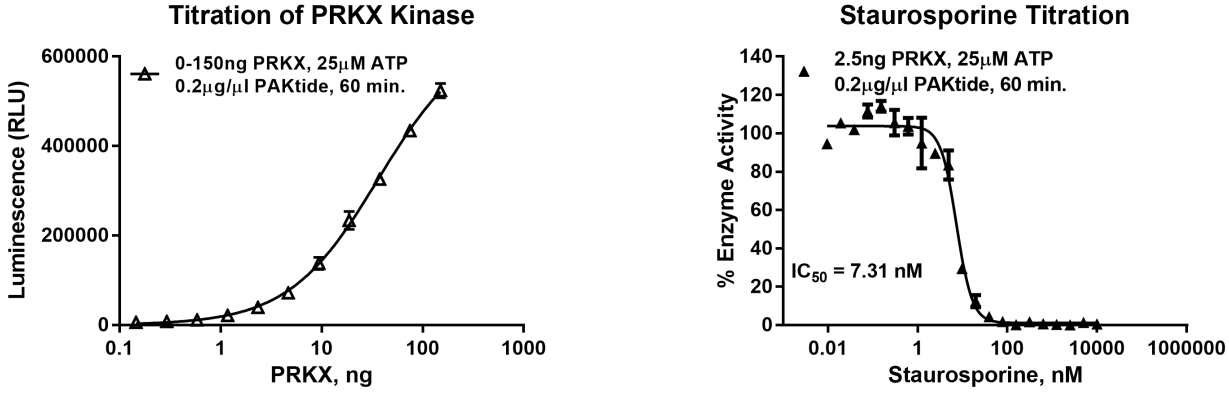

Figure 3. PRKX Kinase Assay Development. (A) PRKX enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2.5ng of PRKX to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PRKX Kinase Enzyme System	10µg	VA7543
	1mg	VA7544
ADP-Glo™ + PRKX Kinase Enzyme System	1 Each	VA7545