ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

PAK6 Kinase Assay

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Scientific Background:

PAK6 is a Ser/Thr protein kinase that is a member of the PAK (p21-activated kinase) family that are implicated in the regulation of a number of cellular processes, including cytoskeleton rearrangement, apoptosis and the MAP kinase signaling pathway. PAK6 is highly expressed in the testis and prostate tissues and interacts with the androgen receptor (AR). In response to androgen, PAK6 cotranslocate into the nucleus with AR (1). PAK6 is weakly expressed in normal prostate epithelium but the expression is increased in primary and metastatic prostate cancer cells and is further increased in tumors that relapse after androgen deprivation therapy (2).

1. Yang F, et al: Androgen receptor specifically interacts with a novel p21-activated kinase, PAK6. J. Biol. Chem. 276: 15345-15353, 2001.

2. Kaur R, et al: Increased PAK6 expression in prostate cancer and identification of PAK6 associated proteins. Prostate. 2008 Oct 1;68(14):1510-6.

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.

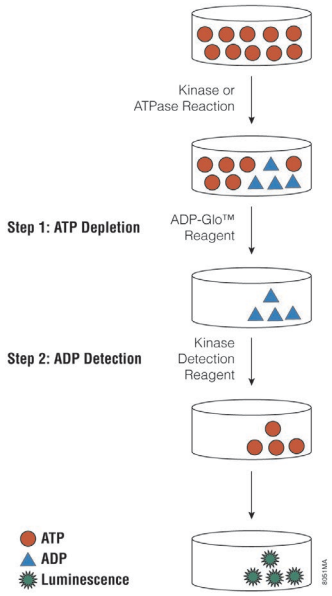

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

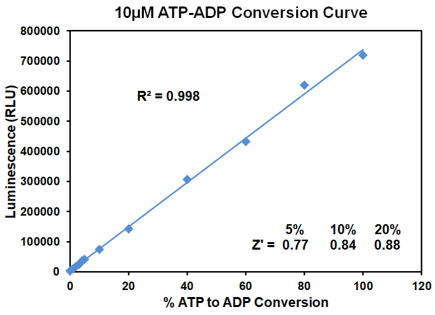

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0
Luminescence	207,106	191,924	185,700	123,276	71,743	34,462	18,970	9,411	5,477	1,605
S/B	129	120	116	77	45	21	12	6	3	1
% Conversion	74	68	66	43	24	11	5	2	0	0

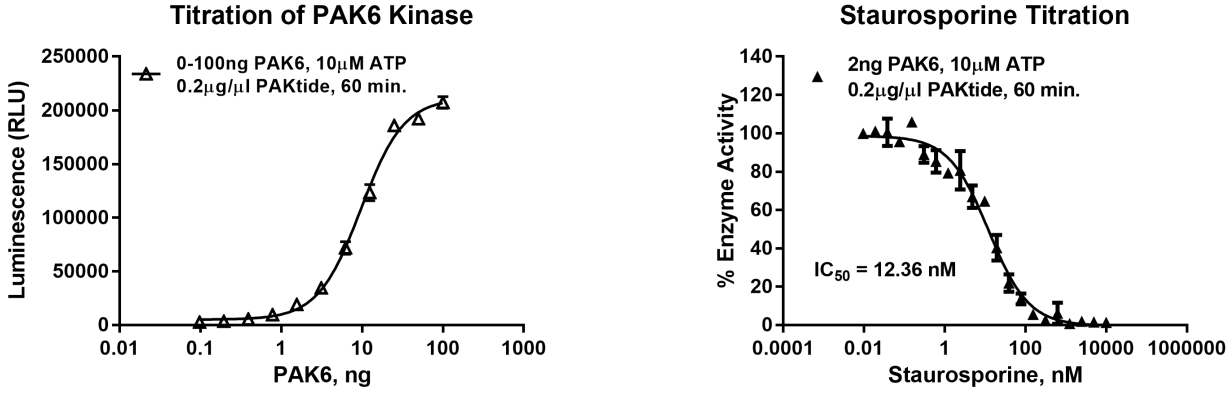

Figure 3. PAK6 Kinase Assay Development. (A) PAK6 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of PAK6 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PAK6 Kinase Enzyme System	10µg	VA7522
	1mg	VA7523
ADP-Glo™ + PAK6 Kinase Enzyme System	1 Each	VA7524