

## PAK1 Kinase Assay

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### Scientific Background:

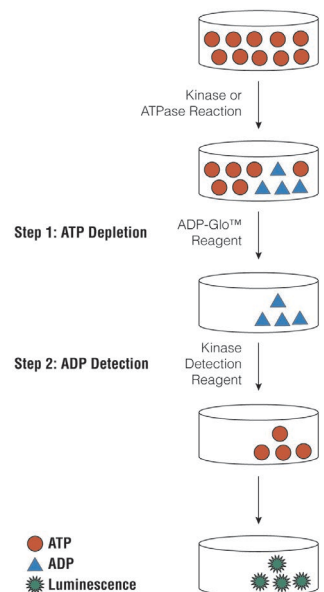
PAK1 is a member of the p21-activated kinases (PAKs) which have been implicated in the regulation of cell morphology, motility and transformation. These serine/threonine kinases are activated by and are effectors of small GTPases, CDC42 and RAC. PAK1 belongs to the Group I PAKs which also includes PAK2 and PAK3 (1). PAK1 is a key regulator of the actin cytoskeleton, adhesion and cell motility. Inactive dimeric PAK1 is mainly cytosolic and interaction with the activators Cdc42-GTP and Rac1-GTP stimulates the kinase at the sites of cellular protrusions forming adhesions to the extracellular matrix (2).

1. Jaffer, Z M. et al: p21-activated kinases: three more join the Pak. *Int J Biochem Cell Biol.* 2002 Jul;34(7):713-7.
2. Parrini, M C. et al: Spatiotemporal regulation of the Pak1 kinase. *Biochem Soc Trans.* 2005 Aug;33(Pt 4):646-8.

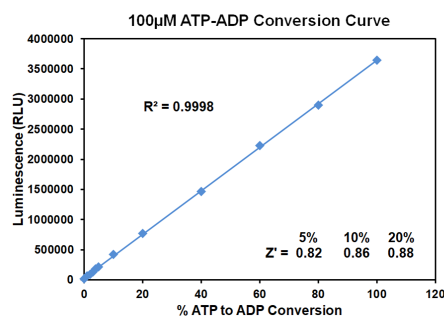
### ADP-Glo™ Kinase Assay

#### 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 100µM 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.

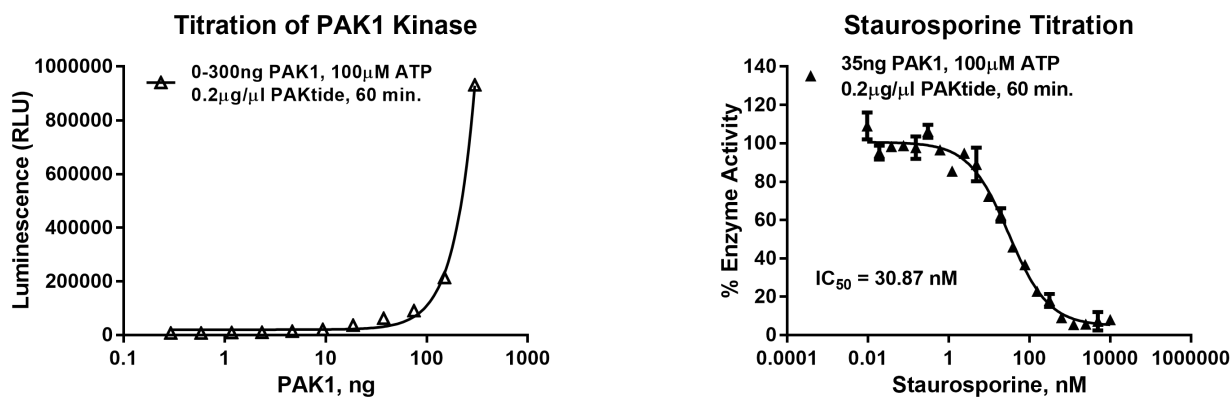
The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: <http://www.promega.com/KESProtocol>

## Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
  - ✓ 1  $\mu$ l of inhibitor or (5% DMSO)
  - ✓ 2  $\mu$ l of enzyme (defined from table 1)
  - ✓ 2  $\mu$ l of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5  $\mu$ l of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10  $\mu$ l 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	300	150	75	37.50	18.75	2.34	0
Luminescence	930,930	213,465	90,539	62,336	36,173	9,457	5,922
S/B	157	36	15	11	6	2	1
% Conversion	25	5	2	1	0	0	0



**Figure 3. PAK1 Kinase Assay Development.** (A) PAK1 enzyme was titrated using 100 $\mu$ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 35ng of PAK1 to determine the potency of the inhibitor ( $IC_{50}$ ).

## Ordering Information:



Products	Size	Cat. #
PAK1 Kinase Enzyme System	10 $\mu$ g	VA7519
	1mg	VA7520
ADP-Glo™ + PAK1 Kinase Enzyme System	1 Each	VA7521