

PAK7 Kinase Assay

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Scientific Background:

PAK7 (also known as PAK5) is a novel human PAK family kinase that contains a CDC42/Rac1 interactive binding (CRIB) motif at the N-terminus and a Ste20-like kinase domain at the C-terminus (1). The p21-activated kinase (PAK) family of protein kinases has recently attracted considerable attention as an effector of Rho family of small G proteins and as an upstream regulator of MAPK signaling pathways during cellular events such as re-arrangement of the cytoskeleton and apoptosis. PAK7 like the other Paks has been implicated in the regulation of cell morphology, motility and transformation.

1. Pandey, A. et al: Cloning and characterization of PAK5, a novel member of mammalian p21-activated kinase-II subfamily that is predominantly expressed in brain. *Oncogene*. 2002 May 30;21(24):3939-48.
- 2.

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.

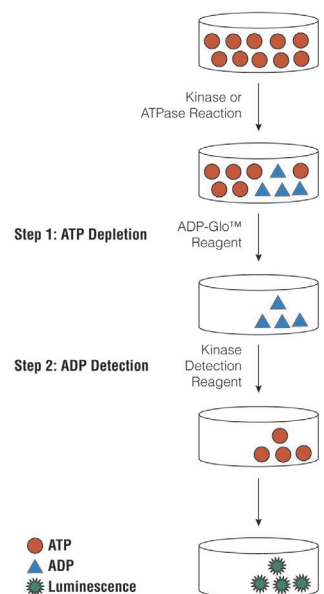


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

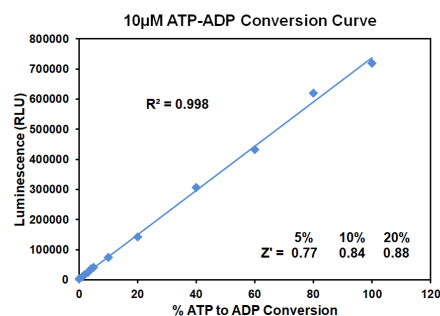


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 μ l of inhibitor or (5% DMSO)
 - ✓ 2 μ l of enzyme (defined from table 1)
 - ✓ 2 μ l of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 μ l of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 μ 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	160	80	40	20	10	5	2.50	0
Luminescence	166,763	72,555	46,406	22,303	13,351	7,110	4,846	2,083
S/B	80	35	22	11	6	3	2	1
% Conversion	59	25	15	6	3	1	0	0

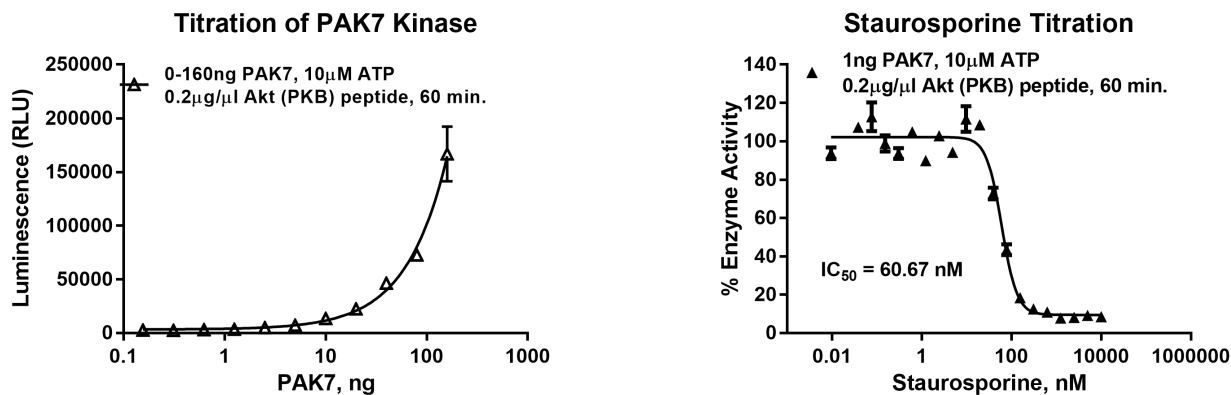


Figure 3. PAK7 Kinase Assay Development. (A) PAK7 enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of PAK7 to determine the potency of the inhibitor (IC₅₀).

Ordering Information:



Products	Size	Cat. #
PAK7 Kinase Enzyme System	10 μ g	VA7525
	1mg	VA7526
ADP-Glo™ + PAK7 Kinase Enzyme System	1 Each	VA7527