ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: PAK3

PAK3 Kinase Assay

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Scientific Background:

PAK3 is a member of the family of serine/threonine p21-activating kinases that serve as targets for the small GTP binding proteins Cdc42 and RAC (1). The PAK family of proteins have been implicated in a wide range of biological activities and are critical effectors that link Rho GTPases to cytoskeleton reorganization and nuclear signaling. PAK3 seems to be necessary for dendritic development and for the rapid cytoskeletal reorganization in dendritic spines associated with synaptic plasticity. A point mutation in PAK3 gene has been linked to nonsyndromic X-linked mental retardation (2).

1. Manser, E. et al: Molecular cloning of a new member of the p21-Cdc42/Rac-activated kinase (PAK) family. J. Biol. Chem. 270: 25070-25078, 1995.

2. Bienvenu, T. et al: Missense mutation in PAK3, R67C, causes X-linked nonspecific mental retardation. Am. J. Med. Genet. 93: 294-298, 2000.

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.

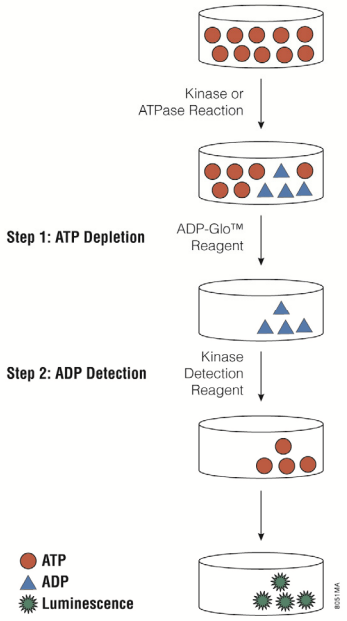


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

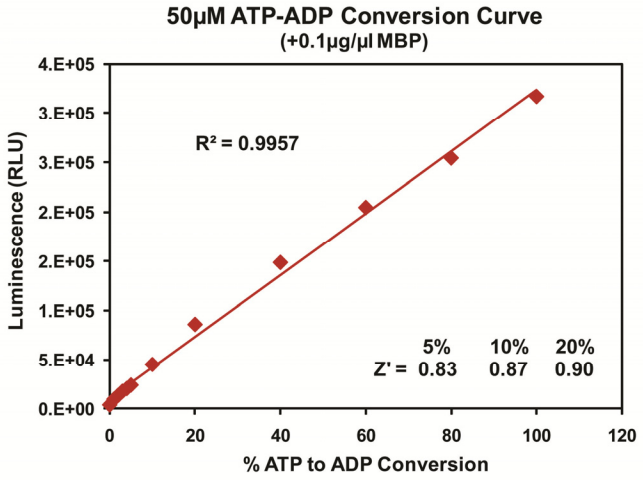


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

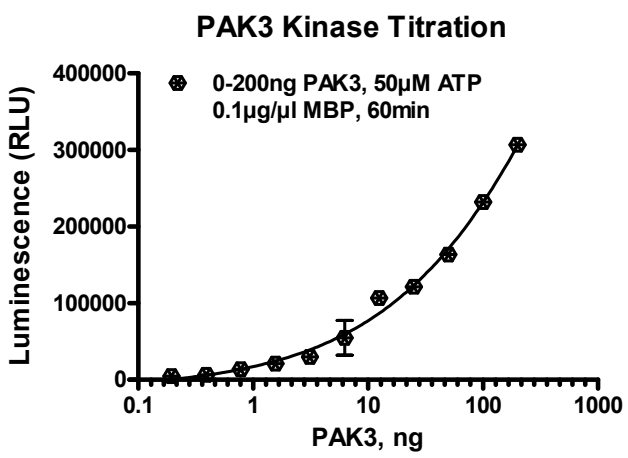
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase 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 60 minutes.
- 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 0.5-1second).

Table 1. PAK3 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.

PAK3, ng	200	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0
RLU	306861	232281	163471	121523	106876	54731	29996	21042	13964	6408	1107
S/B	277	210	148	110	97	49	27	19	13	6	1
% Conversion	95	72	50	37	33	16	9	6	4	1.4	0



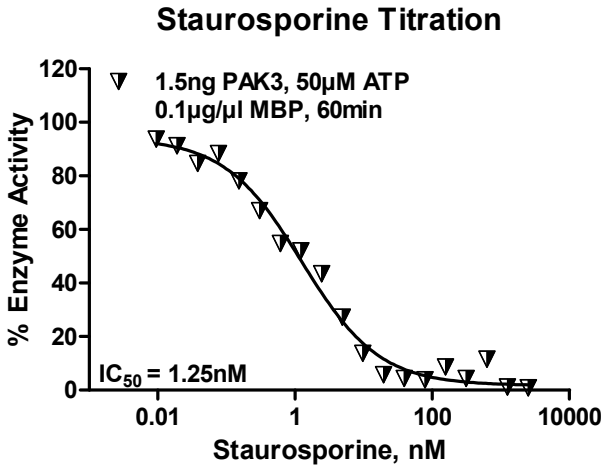


Figure 3. PAK3 Kinase Assay Development. (A) PAK3 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1.5ng of PAK3 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PAK3 Kinase Enzyme System	Promega	V4080
ADP-Glo™ + PAK3 Kinase Enzyme System	Promega	V4081

PAK3 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.