ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: PAK4

PAK4 Kinase Assay

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Scientific Background:

PAK4 is a recently identified 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, cdc 42 and Rac. PAK4 belongs to the Group II PAKs which also includes PAK5 and PAK6. PAK4 has been shown to regulate cell morphology and cytoskeletal organization in mammalian cells.

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. Qu, J. et al: Activated PAK4 regulates cell adhesion and anchorage-independent growth. Mol Cell Biol. 2001 May;21(10):3523-33.

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.

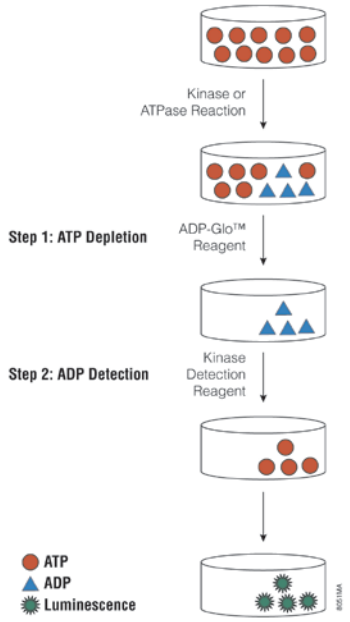

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

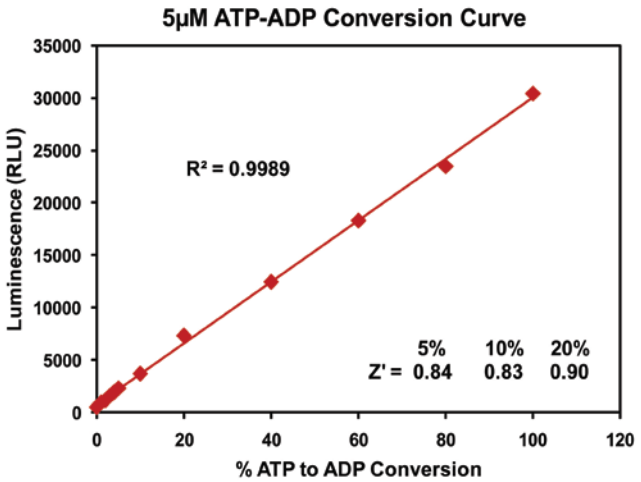

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. PAK4 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.

PAK4, ng	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	14441	13455	12034	9979	6248	3790	2104	1393	998	595
S/B	24.3	22.6	20.2	16.8	10.5	6.4	3.5	2.3	1.7	1
% Conversion	53.0	49.2	43.6	35.5	20.8	11.2	4.6	1.8	0.2	0

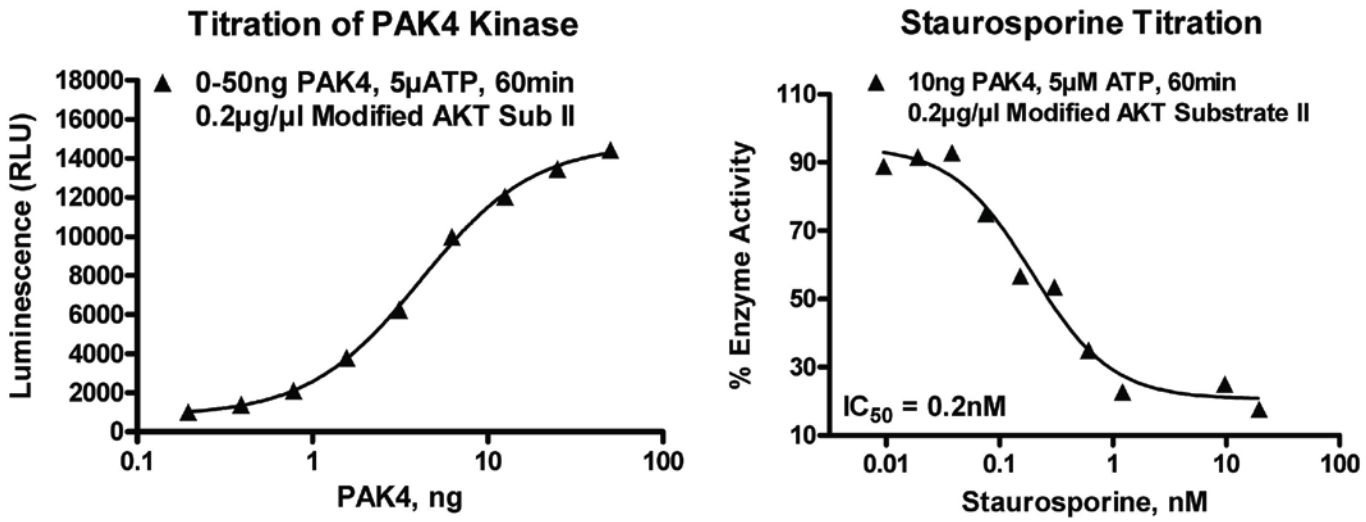

Figure 3. PAK4 Kinase Assay Development. (A) PAK4 enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of PAK4 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PAK4 Kinase Enzyme System	Promega	V3201
ADP-Glo™ + PAK4 Kinase Enzyme System	Promega	V9451

PAK4 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.