ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: PAK1/CDC42

PAK1/CDC42 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.

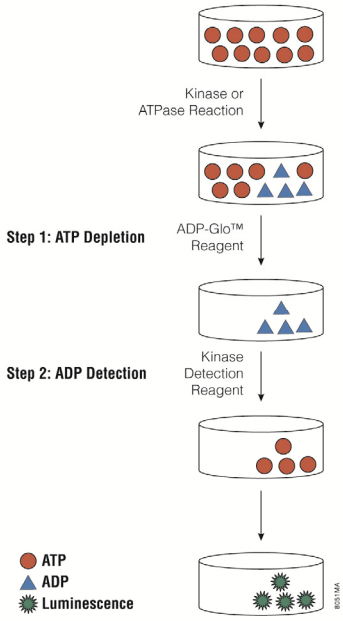


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

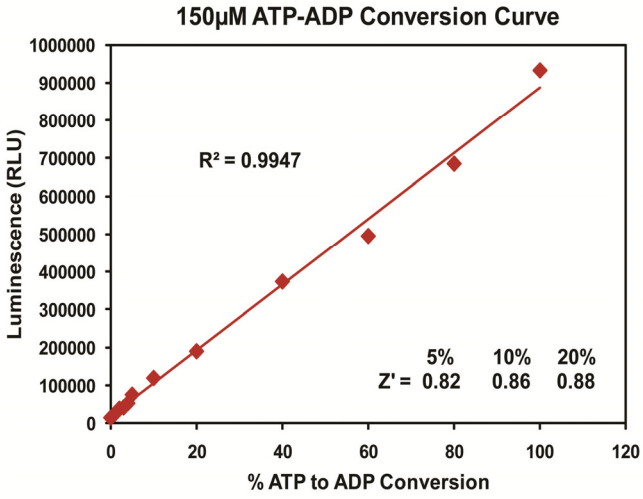


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 150µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

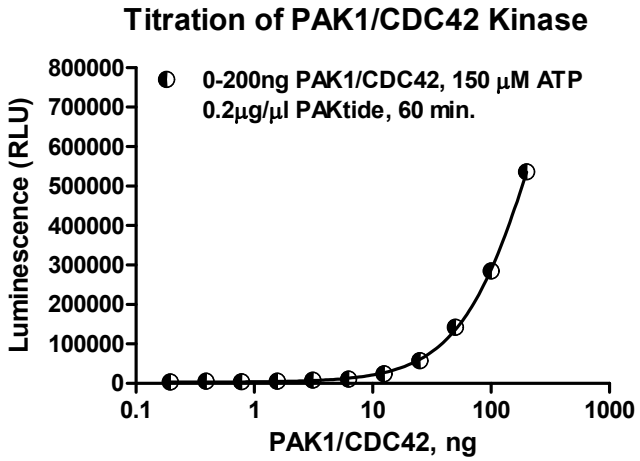
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. PAK1/CDC42 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.

PAK1/CDC42, ng	200	100	50	25	12.5	6.25	3.13	0
RLU	536265	284978	142118	57738	24583	10974	8041	3336
S/B	161	85	43	17	7.4	3.3	2.4	1
% Conversion	77	40	18	6	0.8	0.7	0.6	0



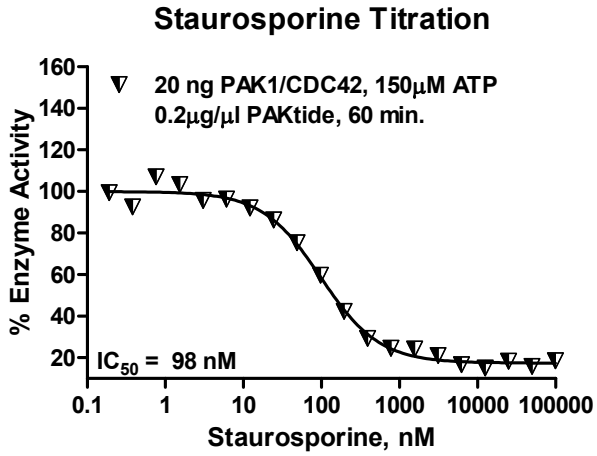


Figure 3. PAK1/CDC42 Kinase Assay Development. **(A) PAK1/CDC42 enzyme was titrated using 150µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 20ng of PAK1/CDC42 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PAK1/CDC42 Kinase Enzyme System	Promega	V4478
ADP-Glo™ + PAK1/CDC42 Kinase Enzyme System	Promega	V4479

PAK1/CDC42 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT; 100µM GTP