PDK1 Kinase Assay

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Scientific Background:

PDK1 (3-phosphoinositide-dependent protein kinase) is activated by the presence of PtdIns(3,4,5)P3 or PtdIns(3,4)P2 (1). PDK1 then activates protein kinase B (PKB) which, in turn, inactivates glycogen synthase kinase-3 (GSK3). The phosphorylation of other proteins by PKB and GSK3 is likely to mediate many of the intracellular actions of insulin. Thus, PDK1 plays a key role in mediating many of the actions of the second messenger(s) PtdIns(3,4, 5)P3 and/or PtdIns(3,4)P2. The human PDK1 is a 556-residue monomeric enzyme comprising of a catalytic domain that is most similar to the PKA, PKB and PKC subfamily of protein kinases.

1. Cohen, P. et al: PDK1, one of the missing links in insulin signal transduction? FEBS Letter. 1997 Jun 23;410(1):3-10. Review.

2. Alessi, DR. et al: Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B alpha. Curr Biol. 1997 Apr 1;7(4):261-9.

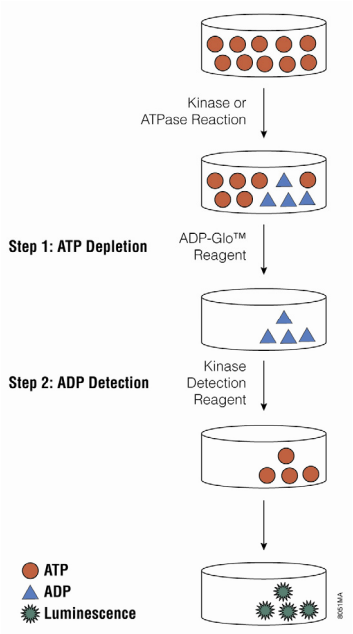

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

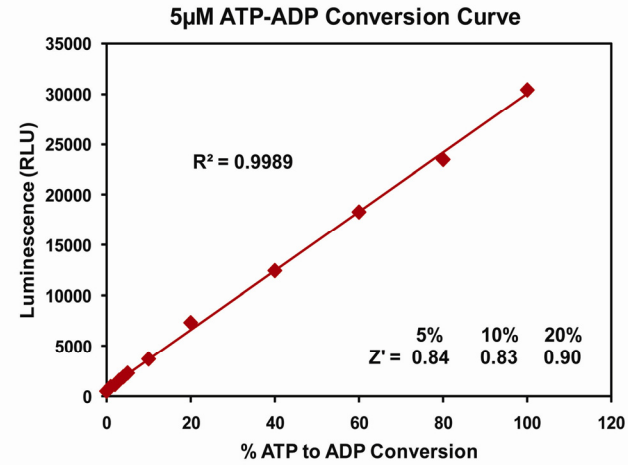

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. PDK1 Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

PDK1, ng	100	50	25	12.5	6.3	3.13	1.56	0.78	0.39	0
RLU	43723	34711	21363	11444	5417	3224	1711	1202	1050	698
S/B	62.6	49.7	30.6	16.4	7.8	4.6	2.5	1.7	1.5	1.0
% Conversion	146	116	70.38	36.4	15.8	8.38	3.21	1.47	0.95	0.00

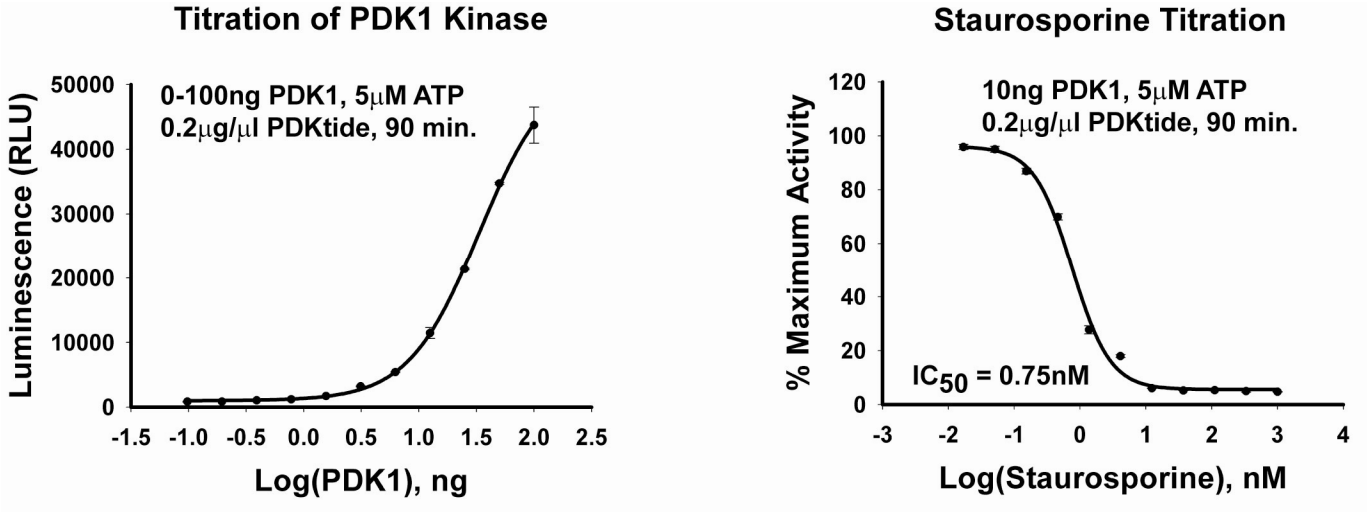

Figure3. PDK1 Kinase Assay Development. (A)PDK1 enzyme was titrated using 5µMATP and the luminescence signal generated from each of the amounts is shown. (B) Staurosporine dose response was created using 10ng of PDK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PDK1 Kinase Enzyme System	Promega	V2761
ADP-Glo + PDK1 Kinase Enzyme System	Promega	V9681

PDK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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