

PKD2 Kinase Assay

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Scientific Background:

PKD2 is a novel phorbol ester- and growth factor-stimulated serine/threonine kinase that contains two cysteine-rich motifs at the N terminus, a pleckstrin homology domain, and a catalytic domain (1). It exhibits the strongest homology to the serine/threonine protein kinases PKD/PKC μ and PKC ν . The PKD family of enzymes have been implicated in very diverse cellular functions, including Golgi organization and plasma membrane directed transport, metastasis, immune responses, apoptosis and cell proliferation (2).

1. Sturany, S. et al: Molecular cloning and characterization of the human protein kinase D2. A novel member of the protein kinase D family of serine threonine kinases. *J Biol Chem.* 2001 Feb 2;276(5):3310-8.
2. Rykx, A. et al: Protein kinase D: a family affair. *FEBS Lett.* 2003 Jul 3;546(1):81-6.

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.

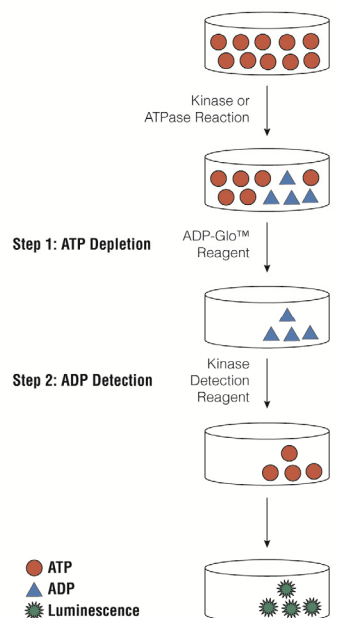


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

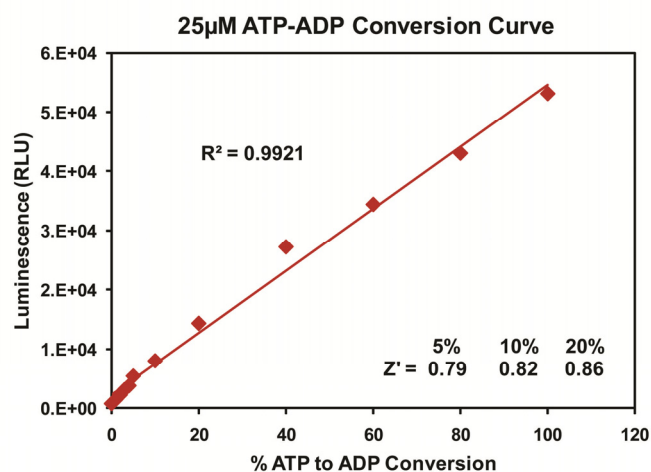


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25 μ M ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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 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 60 minutes.
- 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 0.5-1second).

Table 1. PKD2 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.

PKD2, ng	25	13	6.3	3.1	1.6	0.8	0.4	0.2	0.1	0.05	0
RLU	152766	135571	107715	85908	57170	36541	22429	13336	7103	4399	578
S/B	264	235	186	149	99	63	39	23	12	8	1
% Conversion	100	89	71	56	38	24	15	9	5	3	0

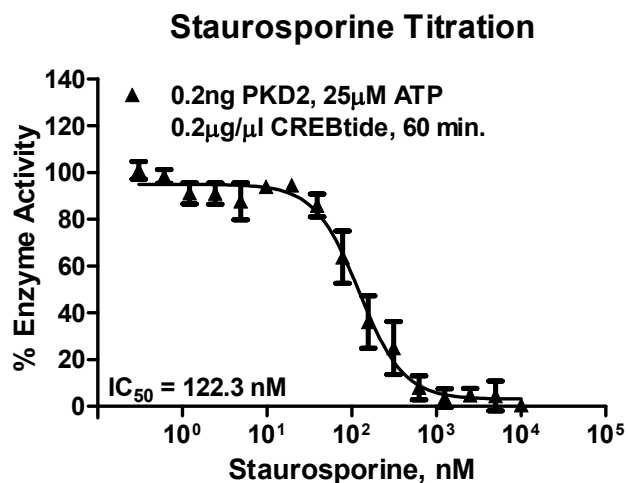
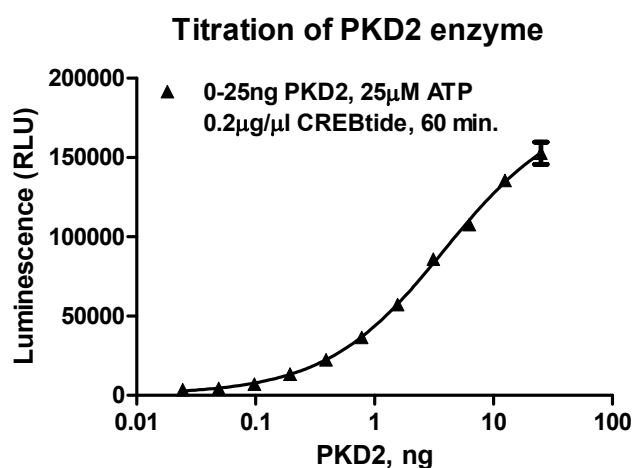


Figure 3. PKD2 Kinase Assay Development. (A) PKD2 enzyme was titrated using 25 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.2ng of PKD2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:		Promega	SignalChem Specialists in Signaling Proteins
Products	Company	Cat.#	
ADP-Glo™ Kinase Assay	Promega	V9101	
PKD2 Kinase Enzyme System	Promega	V4042	
ADP-Glo™ + PKD2 Kinase Enzyme System	Promega	V4043	

PKD2 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50 μ M DTT.