PIM2 Kinase Assay

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Scientific Background:

PIM2 is a serine threonine kinase that is present in all tissues, being most abundant in hematopoietic tissues, spleen, thymus, and peripheral blood leukocytes, as well as in testis, small intestine, and colon (1). It is highly expressed in human leukemic, lymphoma and colorectal adenocarcinoma cell lines. This suggests a role for PIM2 in proliferating cells as well as during meiosis. Similar to PIM1, PIM2 also acts as a pro-survival kinase and BAD protein is a legitimate PIM2 substrate (2).

1. Baytel, D. et al; The human Pim-2 proto-oncogene and its testicular expression. Biochim. Biophys. Acta 1442: 274-285, 1998.

2. Yan, B. et al: The PIM-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. J Biol Chem. 2003 Nov 14;278(46):45358-67.

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.

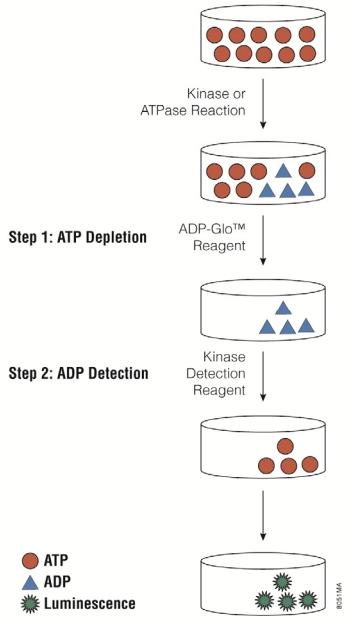


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

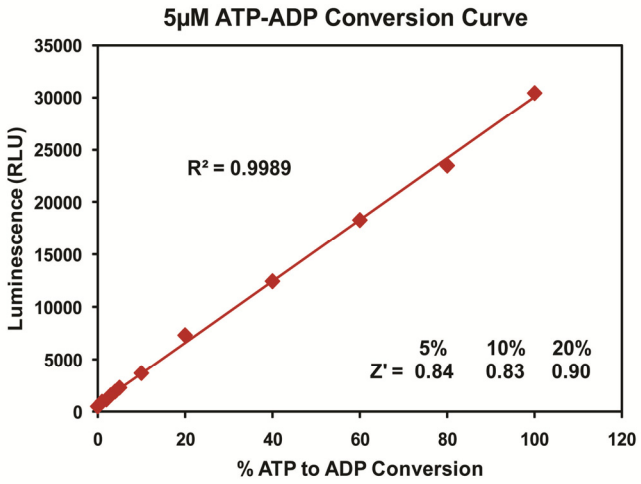


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

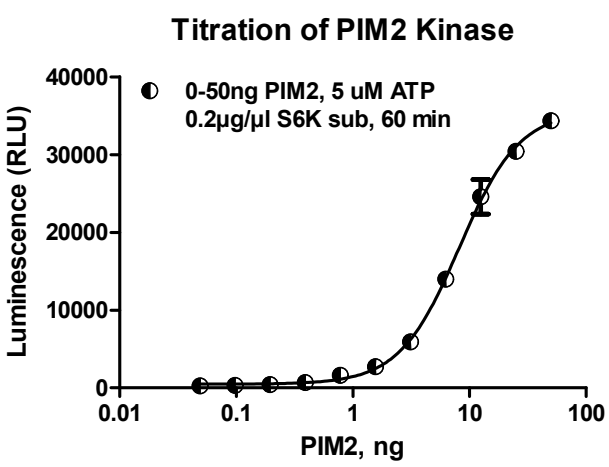
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. PIM2 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.

PIM2, ng	50	25	13	6	3.1	1.6	0.8	0.4	0.2	0
RLU	34356	30401	24576	13968	5904	2706	1631	690	417	196
S/B	175	155	125	71	30	14	8	4	2.1	1
% Conversion	98	87	70	40	16	7	4	1.3	0.5	0



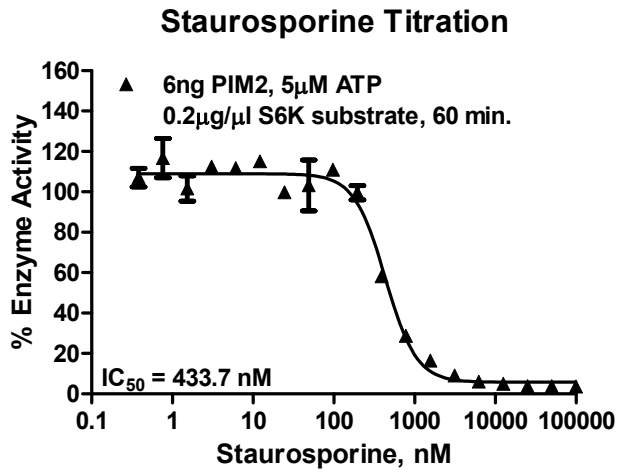


Figure 3. PIM2 Kinase Assay Development. (A) PIM2 enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 6ng of PIM2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PIM2 Kinase Enzyme System	Promega	V4034
ADP-Glo™ + PIM2 Kinase Enzyme System	Promega	V4035

PIM2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.