ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: PIM1

PIM1 Kinase Assay

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Scientific Background:

PIM1 is a proto-oncogene that belongs to a family of serine/threonine protein kinases that are highly conserved through evolution in multicellular organisms. Originally identified from Moloney murine leukemia virus induced T-cell lymphomas in mice, PIM1 is involved in the control of cytokine-mediated cell proliferation, differentiation and survival of lymphoid and myeloid cells as well as others (1). Expression of PIM1 can be stimulated by a variety of growth factors and is regulated at four different levels: transcriptional, post-transcriptional, translational and post-translational (2). Expression of PIM1 is mediated through activation of the JAK/STAT pathway.

1. Meeker, TC. et al: Cloning and characterization of the human PIM-1 gene: a putative oncogene related to the protein kinases. J Cell Biochem. 1987 Oct;35(2):105-12.

2. Friedmann, M. et al: Characterization of the proto-oncogene pim-1: kinase activity and substrate recognition sequence. Arch Biochem Biophys. 1992 Nov 1;298(2):594-601.

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.

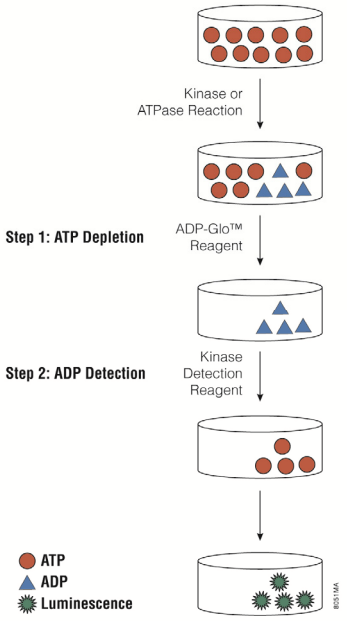

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

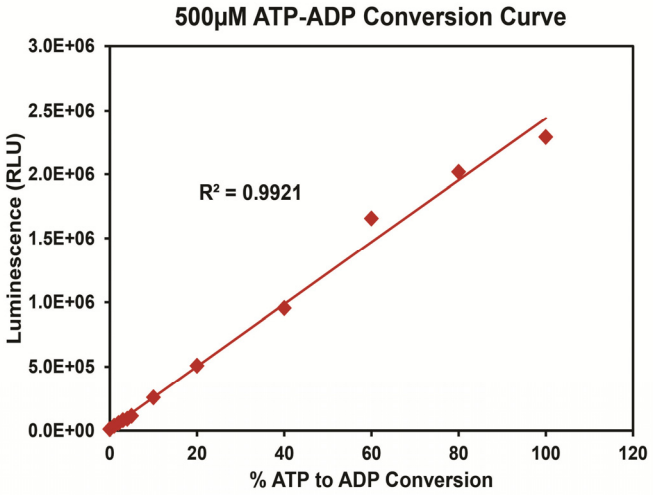

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 500µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction.

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. PIM1 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.

PIM1, ng	200	100	50	25	13	6.3	3.1	1.6	0
RLU	802297	521833	380665	223647	120512	57852	42907	14490	9023
S/B	89	58	42	25	13	6	5	1.6	1
% Conversion	35	22	16	9	4	2	1	0.1	0

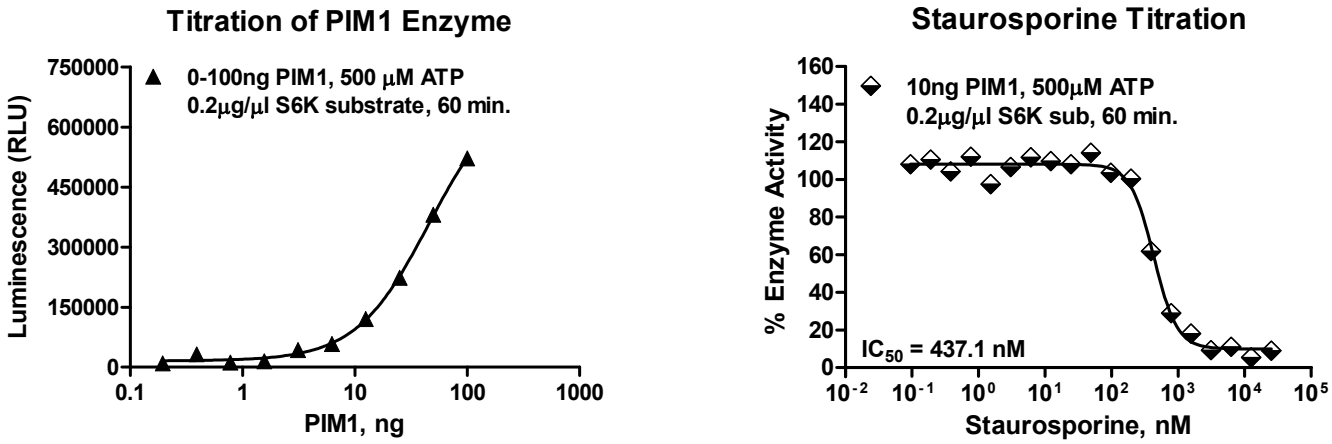

Figure 3. PIM1 Kinase Assay Development. (A) PIM1 enzyme was titrated using 500µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of PIM1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PIM1 Kinase Enzyme System	Promega	V4032
ADP-Glo™ + PIM1 Kinase Enzyme System	Promega	V4033

PIM1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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