HIPK1 Kinase Assay

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Scientific Background:

HIPK1 or homeodomain-interacting protein kinase 1 is a ser/thr protein kinase and a member of the HIPK family. HIPK 1 is a nuclear kinase that phosphorylates homeodomain transcription factors. HIPK1 phosphorylates DAXX and this leads to its relocalization and subsequent decrease in transcriptional repression activity (1). HIPK1also interacts with p53 and phosphorylates it on serine residues. HIPK 1 expression is elevated in breast cancer cell lines and embryonic fibroblasts from HIPK 1-null mice show more susceptibility to apoptosis induced by DNA damage (2).

1. Ecsedy, J A. et al: Homeodomain-interacting protein kinase 1 modulates Daxx localization, phosphorylation, and transcriptional activity. Mol Cell Biol. 2003 Feb;23(3):950-60.

2. Kondo, S. et al: Characterization of cells and gene-targeted mice deficient for the p53-binding kinase homeodomain-interacting protein kinase 1 (HIPK1). Proc Natl Acad Sci U S A. 2003 Apr 29;100(9):5431-6.

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.

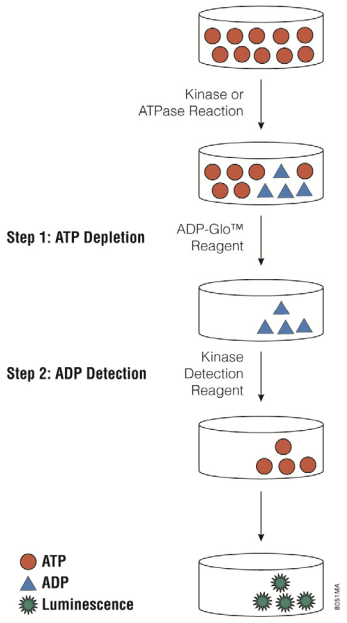

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

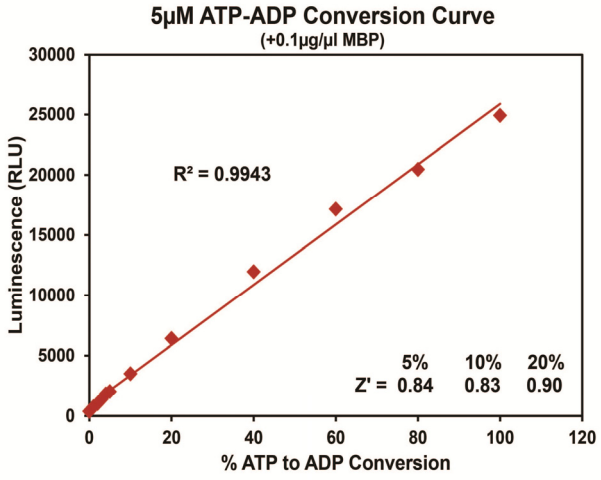

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. HIPK1 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.

HIPK1, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
RLU	33024	32051	31051	29644	28517	18995	7344	3871	1367	806	302
S/B	109	106	103	98	94	63	24	13	5	3	1
% Conversion	94	91	88	84	81	54	20	10	3	1.2	0

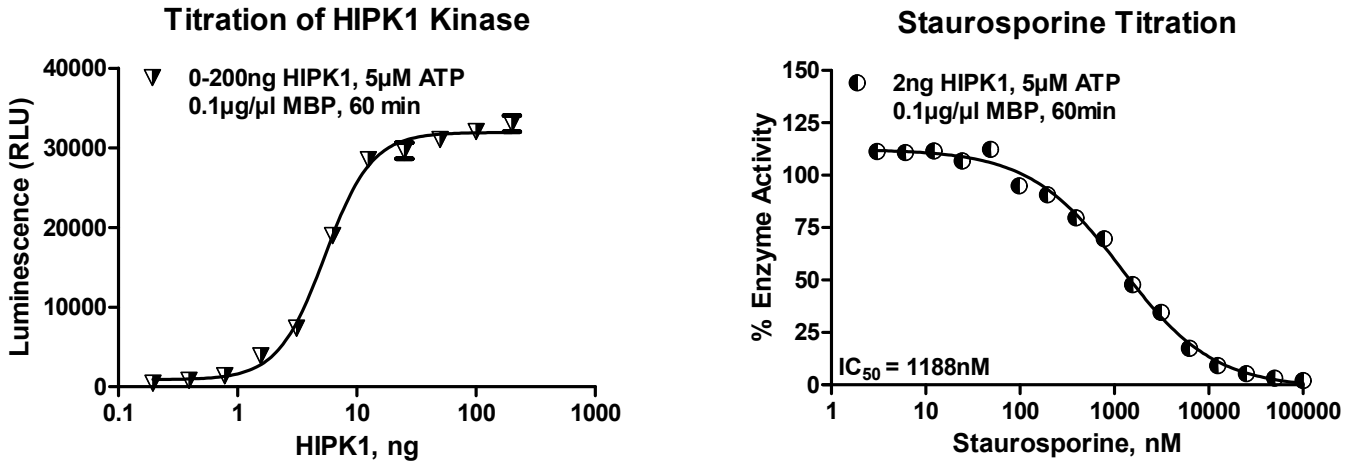

Figure 3. HIPK1 Kinase Assay Development. (A) HIPK1 enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of HIPK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
HIPK1 Kinase Enzyme System	Promega	V4066
ADP-Glo™ + HIPK1 Kinase Enzyme System	Promega	V4067

HIPK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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