ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: HIPK3

HIPK3 Kinase Assay

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Scientific Background:

HIPK3 (homeodomain interacting protein kinase 3) is a member of the HIPK family and is involved in apoptosis (1). JNK regulates the expression of HIPK3 in prostate cancer cells and this contributes to increased resistance to Fas receptor-mediated apoptosis by reducing the interaction between FADD and caspase-8 (2). HIPK3 has been reported to phosphorylate FADD and is implicated in multidrug resistance in a number of tumors. HIPK3 increases transcription factor SF-1 activity leading to increased steroidogenic gene expression in response to cAMP signaling.

1. Begley, D.A. et al: Identification and sequence of human PKY, a putative kinase with increased expression in multidrug-resistant cells, with homology to yeast protein kinase Yak1. Gene, 1997; 200 (1-2): 35–43.

2. Curtin, J F. et al: JNK regulates HIPK3 expression and promotes resistance to Fas-mediated apoptosis in DU 145 prostate carcinoma cells. J Biol Chem. 2004 Apr 23;279(17):17090-100.

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.

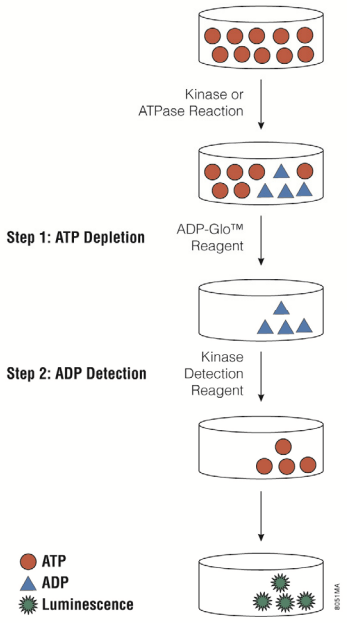


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

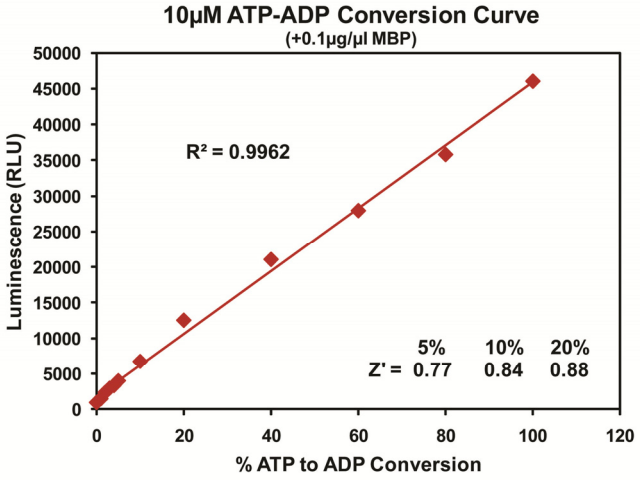


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

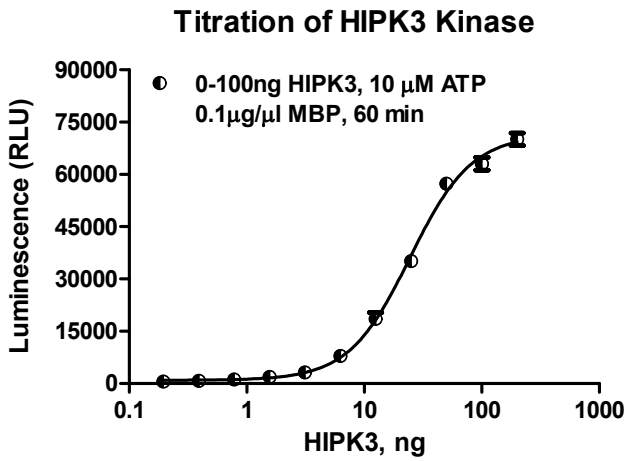
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. HIPK3 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.

HIPK3, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
RLU	68278	63044	57302	35153	18596	7948	3164	1890	1159	767	378
S/B	181	167	152	93	49	21	8	5	3.1	2.0	1
% Conversion	100	93	85	52	27	11	4	2	0.8	0.2	0



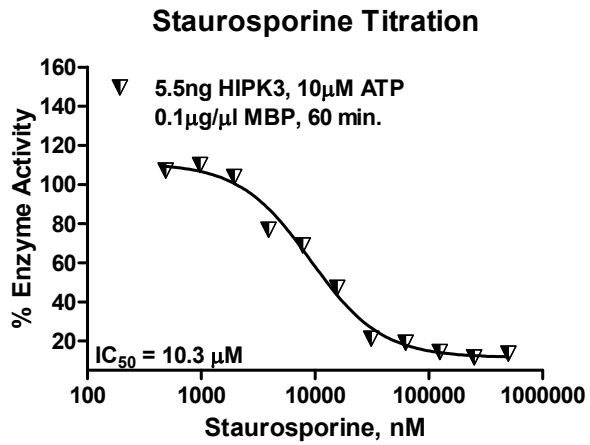


Figure 3. HIPK3 Kinase Assay Development. (A) HIPK3 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 5.5ng of HIPK3 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
HIPK3 Kinase Enzyme System	Promega	V4164
ADP-Glo™ + HIPK3 Kinase Enzyme System	Promega	V4165

HIPK3 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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