ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: HPK1

HPK1 Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

HPK1 or Hematopoietic progenitor kinase 1 is a hematopoietic cell-restricted member of the Ste20 serine/threonine kinase super family. HPK1 is also known as mitogen-activated protein kinase kinase kinase kinase 1(MAP4K1). HPK1 is a tissue-specific upstream activator of the MEKK/JNK/SAPK signaling pathway (1). HPK1 diminishes T cell receptor (TCR) signaling activity and T cell proliferation by phosphorylating the adaptor protein SLP-76 (2).

1. Hu, M. et.al: Human HPK1, a novel human hematopoietic progenitor kinase that activates the JNK/SAPK kinase cascade. Genes Dev. 10: 2251-2264, 1996.

2. Shui JR. et.al: Hematopoietic progenitor kinase 1 negatively regulates T cell receptor signaling and T cell-mediated immune responses. Nat. Immunol. 8, 84 - 91 (2007).

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.

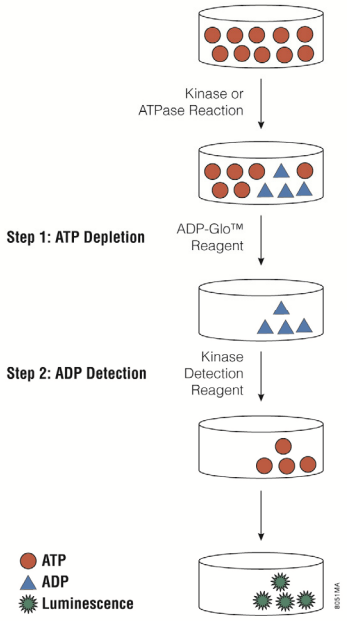

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

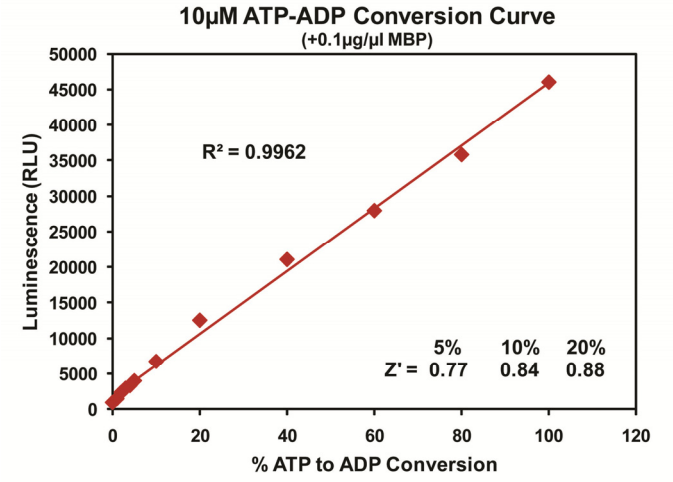

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. HPK1 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.

HPK1, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
RLU	58375	56559	46974	33271	21297	11416	7197	3729	1936	1068	424
S/B	138	133	111	78	50	27	17	9	4.6	2.5	1
% Conversion	98	95	79	55	35	18	10	4	1.3	0.6	0

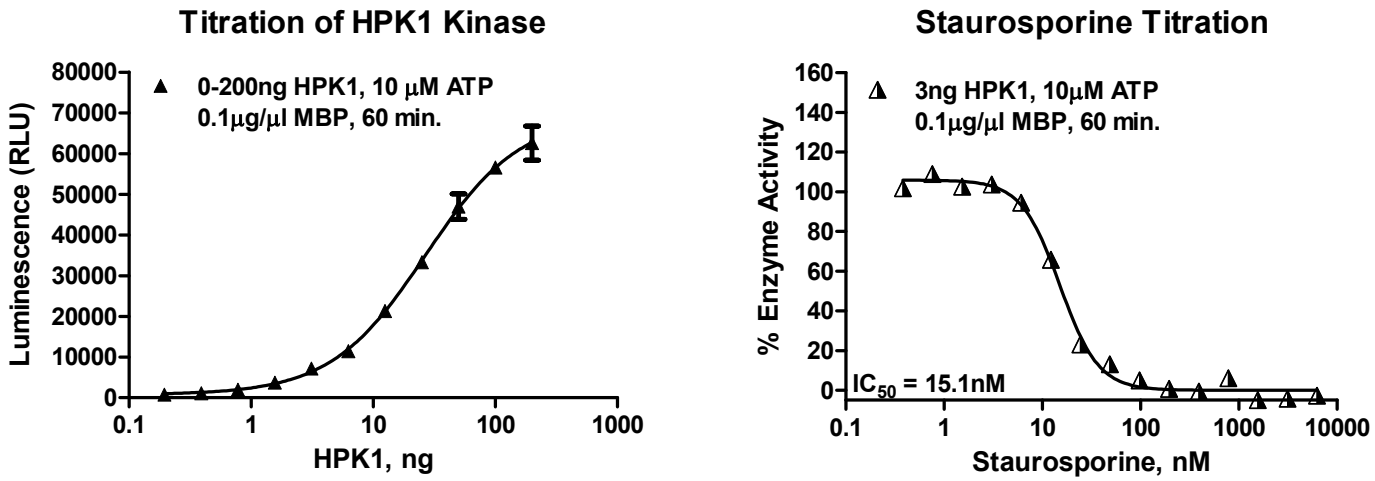

Figure 3. HPK1 Kinase Assay Development. (A) HPK1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 3ng of HPK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
HPK1 Kinase Enzyme System	Promega	V4098
ADP-Glo™ + HPK1 Kinase Enzyme System	Promega	V4099

HPK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
promega.com