ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: MAPKAPK3

MAPKAPK3 Kinase Assay

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Scientific Background:

MAPKAPK3 is closely related to MAPKAPK2 sharing 72% nucleotide and 75% amino acid identity (1). MAPKAPK3 is activated by growth inducers and stress stimulation of cells. In vitro studies demonstrated that ERK, p38 MAP kinase and Jun N-terminal kinase were all able to phosphorylate and activate this kinase, which suggested the role of MAPKAPK3 as an integrative element of signaling in both mitogen and stress responses (2).

1. Sithanandam, G. et al: 3pK a new mitogen-activated protein kinase-activated protein kinase located in the small cell lung cancer tumor suppressor gene region. Molec. Cell. Biol. 16: 868-876, 1996.

2. Ludwig, S. et al: 3pK, a novel mitogen-activated protein (MAP) kinase-activated protein kinase, is targeted by three MAP kinase pathways. Molec. Cell. Biol. 16: 6687-6697, 1996.

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.

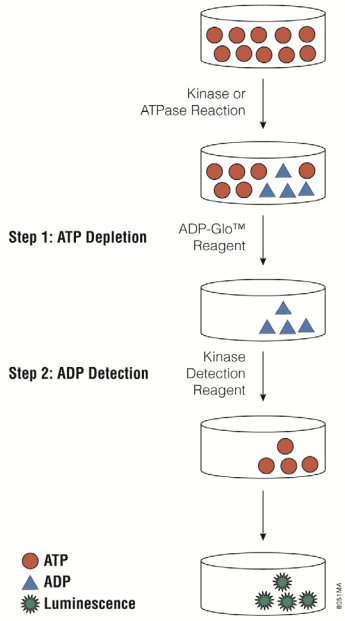


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

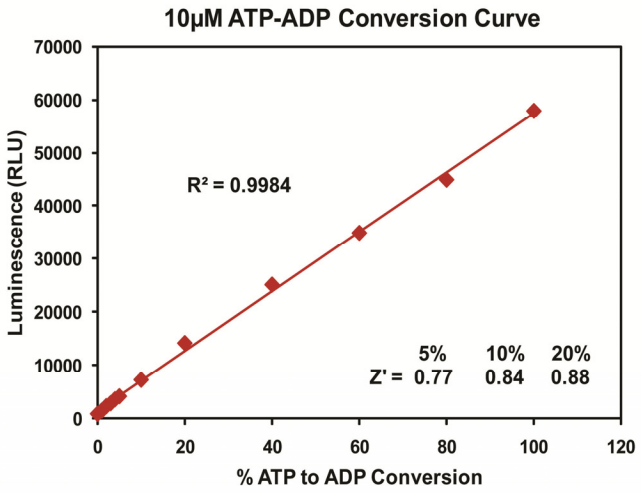


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

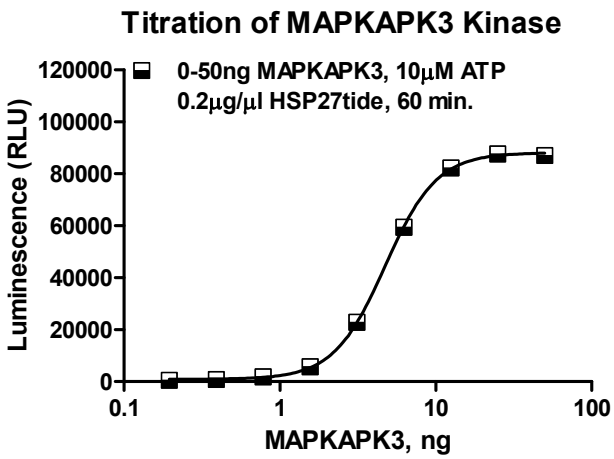
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. MAPKAPK3 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.

MAPKAPK3, ng	50	25	13	6.25	3.13	1.56	0.78	0.39	0.20	0
RLU	87031	87673	82252	59464	22819	5738	1873	885	541	318
S/B	274	276	259	187	72	18	6	3	2	1
% Conversion	97	98	92	66	25	6	2	0.5	0.1	0



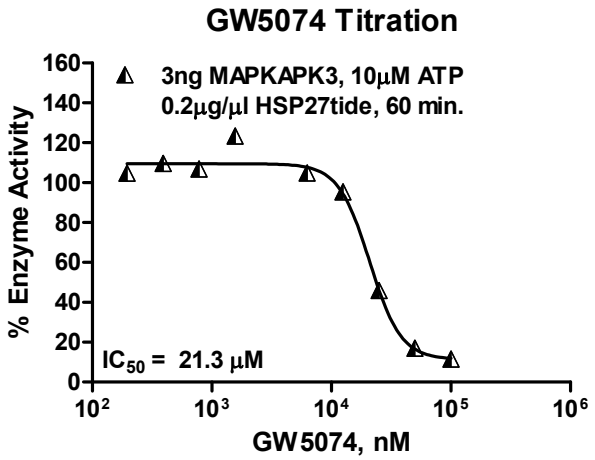


Figure 3. MAPKAPK3 Kinase Assay Development. **(A) MAPKAPK3 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) GW5074 dose response was created using 3ng of MAPKAPK3 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
MAPKAPK3 Kinase Enzyme System	Promega	V4026
ADP-Glo™ + MAPKAPK3 Kinase Enzyme System	Promega	V4027

MAPKAPK3 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.