MLK2 Kinase Assay

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Scientific Background:

MLK2 or mixed-lineage kinase 2 is a member of the serine/threonine kinase family, which activates MAPK8 JNK and MKK4/SEK1. MLK2 itself can be phoshorylated, and activated by JNK kinases. MLK2 is also known as mitogen-activated protein kinase kinase kinase 10 (MAP3K10). MLK2 functions preferentially on the JNK signaling pathway which is involved in nerve growth factor (NGF) induced neuronal apoptosis. MLK2 was isolated as a cDNA fragment from MKN28 gastric cancer cell library using degenerate PCR (1). MLK2 is highly expressed in brain, skeletal muscle, and testis (2).

1. Katoh, M. et.al: Cloning and characterization of MST, a novel (putative) serine/threonine kinase with SH3 domain. Oncogene 10: 1447-1451, 1995.

2. Dorow, D. S. et.al: complete nucleotide sequence, expression, and chromosomal localisation of human mixed-lineage kinase 2. Europ. J. Biochem. 234: 492-500, 1995.

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.

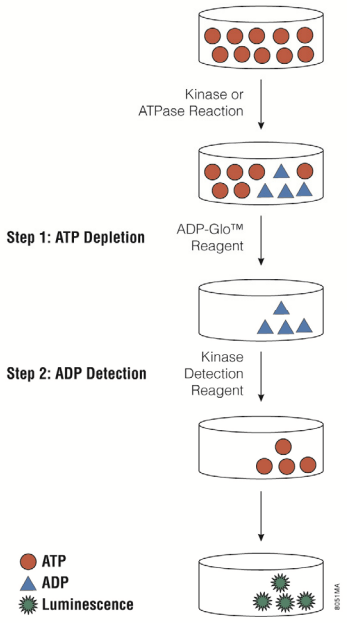


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

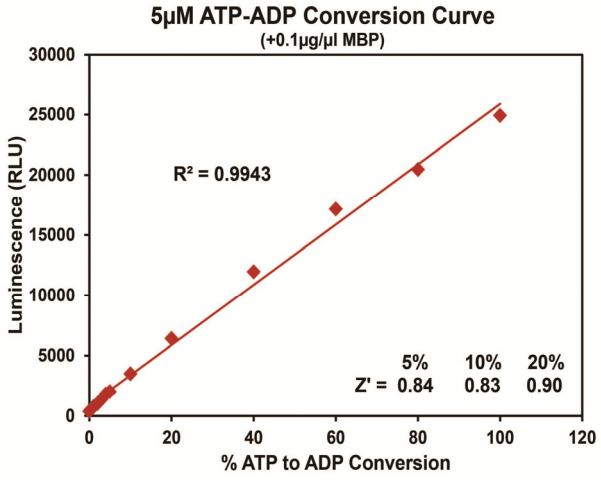


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

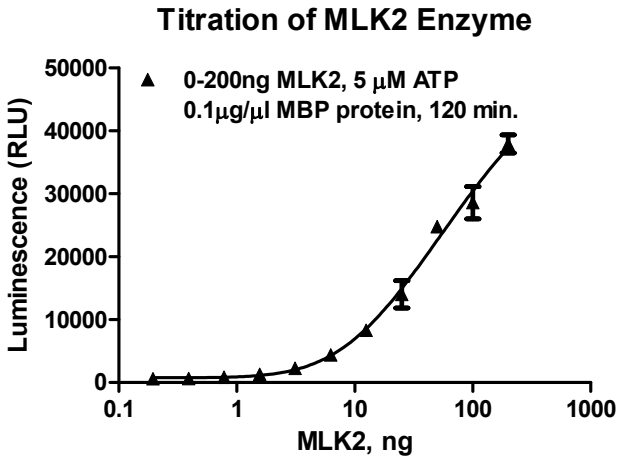
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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 120 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. MLK2 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.

MLK2, ng	200	100	50	25	12.5	6.25	3.13	1.56	0
RLU	37912	28584	24763	14030	8320	4381	2268	1295	490
S/B	77	58	51	29	17	9	4.6	2.6	1
% Conversion	92	69	59	32	18	8	3.2	0.8	0



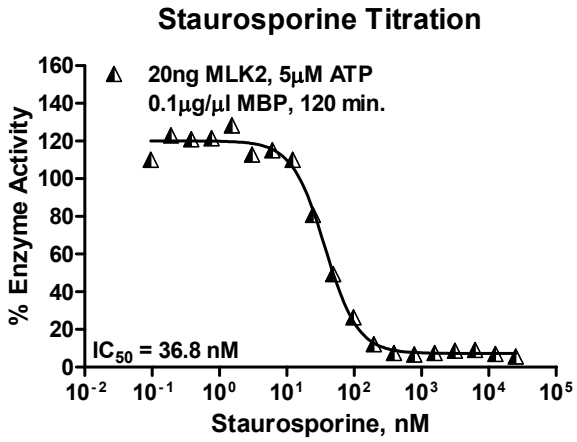


Figure 3. MLK2 Kinase Assay Development. (A) MLK2 enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 20ng of MLK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
MLK2 Kinase Enzyme System	Promega	V4476
ADP-Glo™ + MLK2 Kinase Enzyme System	Promega	V4477

MLK2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.