Promega

# MLK1 Kinase Assay

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## Scientific Background:

MLK1 or Mixed-Lineage Kinase 1 is a mitogen-activated protein kinase kinase kinase capable of activating the c-Jun NH(2)-terminal kinase (JNK) pathway. The catalytic domain of MLK1 has amino acid sequence similarity to both the tyr-specific and the ser/thr-specific kinase classes. In addition, MLK1 contain 2 leu/ile-zipper motifs and a basic sequence near its C-termini (1). MLK1 is threonine (and possibly serine) phosphorylated at multiple sites in the activation loop, with phosphorylation of Thr312 required for full activation (2).

1. Dorow, D S. et al: Identification of a new family of human epithelial protein kinases containing two leucine/isoleucine-zipper domains. Europ. J. Biochem. 213: 701-710, 1993.

2. Durkin, J T. et al: Phosphoregulation of mixed-lineage kinase 1 activity by multiple phosphorylation in the activation loop. Biochemistry. 2004 Dec 28;43(51):16348-55.

## 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.

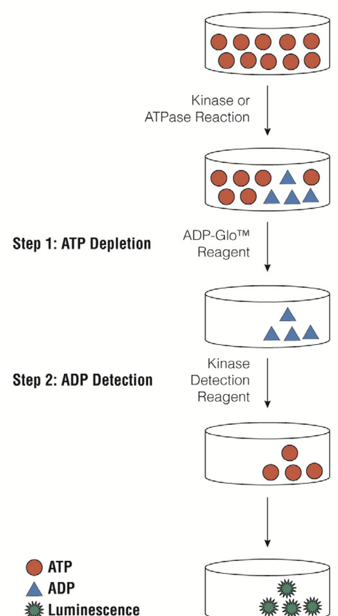



**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

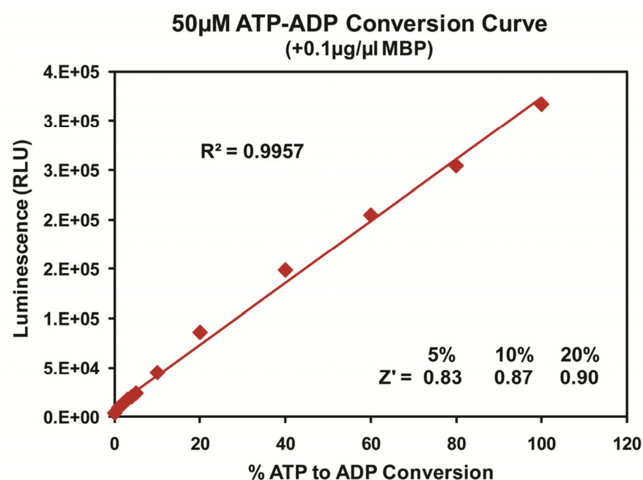
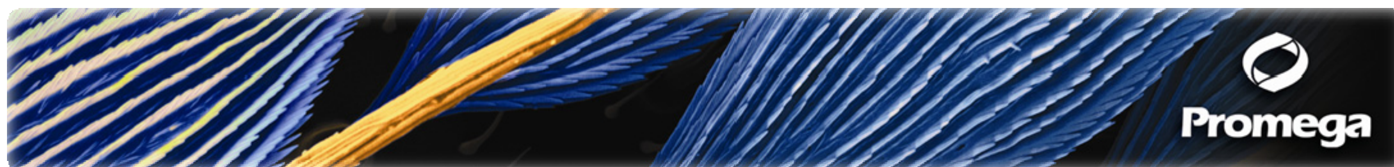



**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



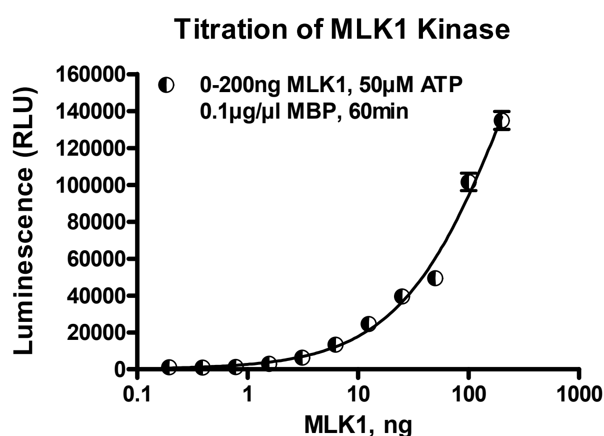

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

## 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. MLK1 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.

| MLK1, ng | 200 | 100 | 50 | 25 | 13 | 6.3 | 3.1 | 1.6 | 0 |
|---|---|---|---|---|---|---|---|---|---|
| RLU | 134909 | 101573 | 49421 | 39485 | 24556 | 13388 | 6341 | 3032 | 1021 |
| S/B | 132 | 99 | 48 | 39 | 24 | 13 | 6 | 3.0 | 1 |
| % Conversion | 54 | 41 | 20 | 16 | 10 | 5 | 2 | 0.85 | 0 |



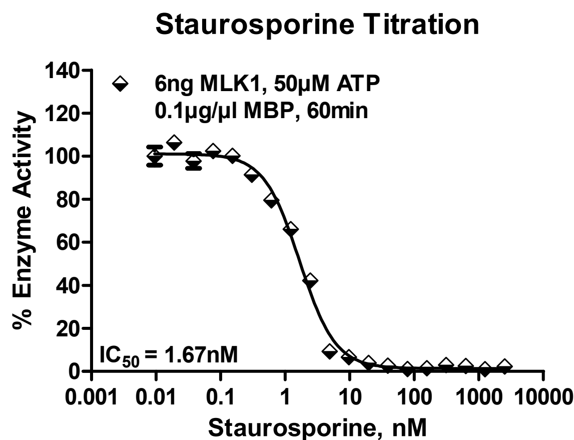




**Figure 3. MLK1 Kinase Assay Development.** (A) MLK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 6ng of MLK1 to determine the potency of the inhibitor ($IC_{50}$).

**Assay Components and Ordering Information:**

Promega SignalChem Specialists in Signaling Proteins

| Products | Company | Cat.# |
|---|---|---|
| ADP-Glo™ Kinase Assay | Promega | V9101 |
| MLK1 Kinase Enzyme System | Promega | V4072 |
| ADP-Glo™ + MLK1 Kinase Enzyme System | Promega | V4073 |

MLK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2.5mM $MnCl_2$; 50µM DTT.