ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

MLK4 Kinase Assay

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Scientific Background:

MLK4 or mixed lineage kinase 4 belongs to the superfamily of MAP kinase kinase kinases (MAP3K1) which contain both ser/thr and tyr kinases activity in their catalytic domains. The structure of this kinase family incorporates an N-terminal Src homology (SH3) domain, followed by the kinase domain, a leucine zipper region, and a CDC42 /RAC -interactive binding (CRIB) motif, but divergent C-terminal regions. MLK4 is highly expressed in kidney and pancreas (1). MLK4 is a negative regulator of TLR4 (2).

1. Nagase, T. et.al;: Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Res. 8: 85-95, 2001.

2. Seit-Nebi, A. et.al: MLK4 has negative effect on TLR4 signaling. Cell. Molec. Immun. 9: 27-33, 2012.

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.

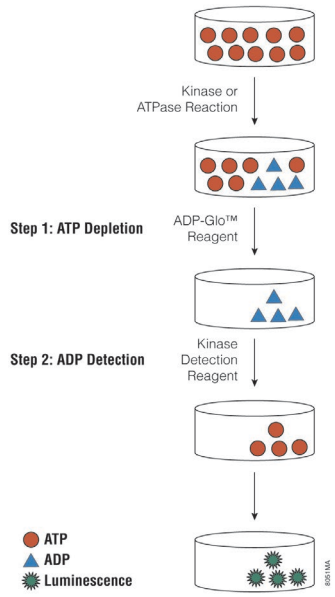

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

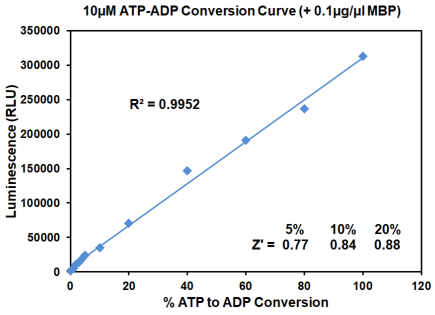

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	300	150	75	37.50	18.75	4.69	2.34	0
Luminescence	170,280	76,669	38,592	15,786	8,252	2,019	1,352	780
S/B	218	98	49	20	11	3	2	1
% Conversion	57	25	12	4	2	0	0	0

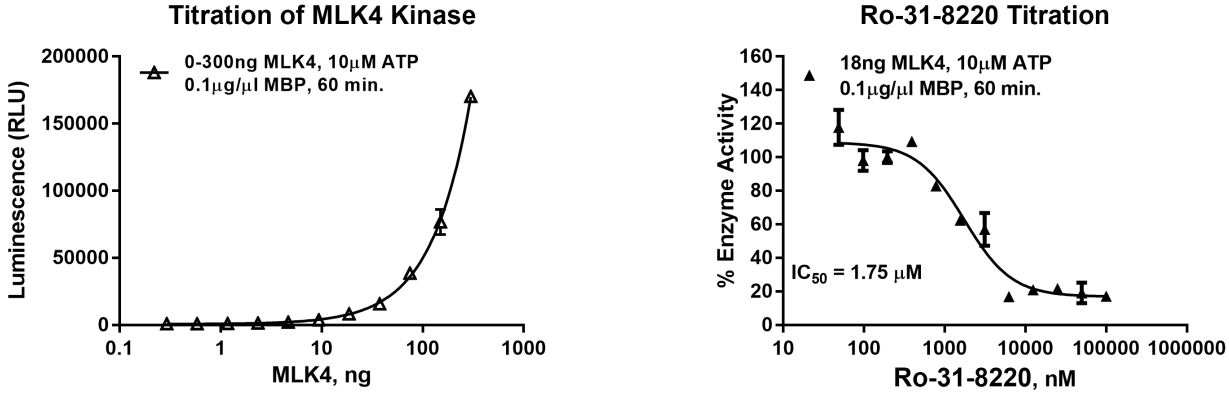

Figure 3. MLK4 Kinase Assay Development. (A) MLK4 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 18ng of MLK4 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MLK4 Kinase Enzyme System	10µg	VA7504
	1mg	VA7505
ADP-Glo™ + MLK4 Kinase Enzyme System	1 Each	VA7506