ADP-Glo™ Kinase Assay Application Note

Dual-Specificity Kinase Series

MEK4 (MAP2K4) Kinase Assay

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Scientific Background:

Mitogen-activated protein kinase kinase 4 (MEK4 or MKK4) is dual specificity kinase of the STE7 family that phosphorylates and activates JNK1 and JNK2 as well as p38. MKK4 preferentially phosphorylates the tyrosine residue of JNKs. Mediates a variety of physiological and pathophysiological processes such as responses to cellular stresses and inflammatory cytokines. MKK4 is important for carcinogenesis, a loss-off-function mutation in MKK4 was found in lung and pancreatic tumors. Conversely, a pro-oncogenic role was also found for MKK4.

1. Lin A, et al. Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. Science. 268: 286-90, 1995.

2. Wang L, et al. Evidence of MKK4 pro-oncogenic activity in breast and pancreatic tumors. Oncogene. 23:5978-85, 2004.

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.

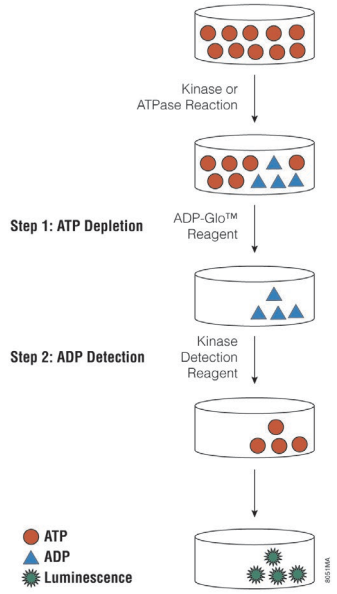

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

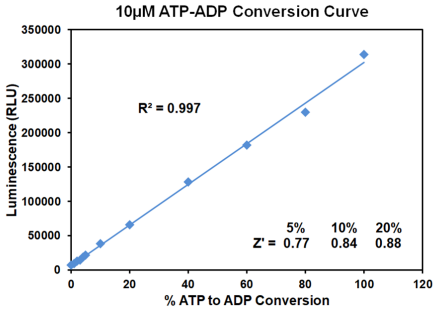

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	140	70	35	17.50	8.75	4.38	2.19	1.09	0
Luminescence	216,266	185,708	179,710	176,449	142,013	79,817	36,394	20,487	10,922
S/B	20	17	16	16	13	7	3	2	1
% Conversion	71	60	58	57	46	25	10	5	0

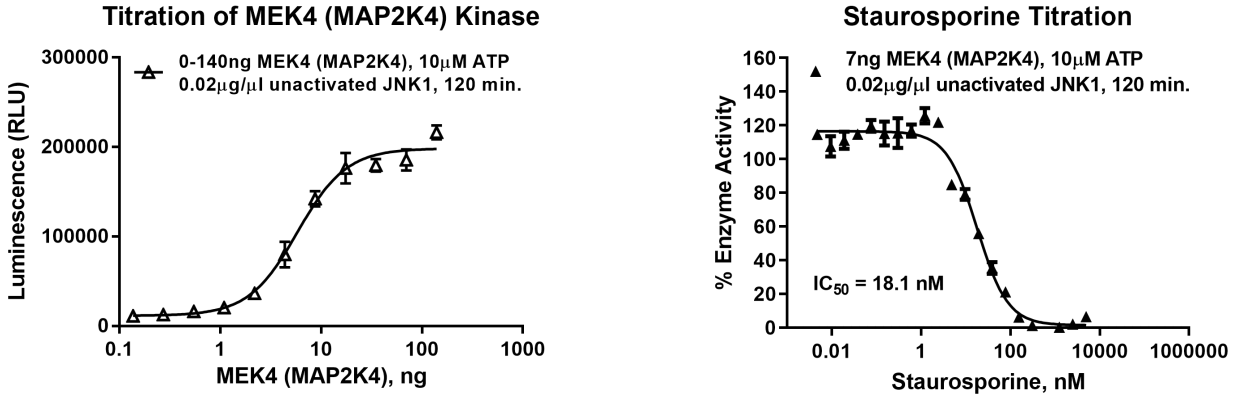

Figure 3. MEK4 (MAP2K4) Kinase Assay Development. (A) MEK4 (MAP2K4) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 7ng of MEK4 (MAP2K4) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MEK4 (MAP2K4) Kinase Enzyme System	10µg	VA7219
	1mg	VA7220
ADP-Glo™ + MEK4 (MAP2K4) Kinase Enzyme System	1 Each	VA7221