ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

MEK1 Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

MEK1 is a member of the dual specificity protein kinase family that acts as a mitogen-activated protein kinase (MAPK) kinase. MEK1 lies upstream of MAPK/ERK and stimulates the enzymatic activity of MAPK/ERK upon a wide variety of extra- and intracellular signals. As an essential component of MAPK/ERK signal transduction pathway, MEK1 is involved in many cellular processes such as proliferation, differentiation, transcription regulation and development (1). Constitutive activation of MEK1 results in cellular transformation. Thus, MEK1 represents a likely target for pharmacologic intervention in proliferative disease such as cancer (2).

1. Seger, R. et al: The MAPK signaling cascade. FASEB J. 9: 726-735, 1995.

2. Sebolt-Leopold, J S. et al: Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. Nature Med. 5: 810-816, 1999.

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.

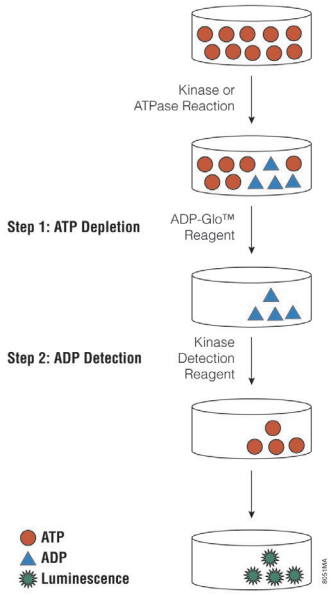

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

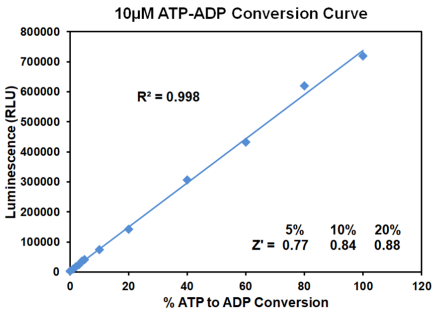

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	80	40	20	10	5	2.50	1.25	0.63	0
Luminescence	334,587	206,480	109,770	53,306	25,042	11,850	6,207	4,489	2,428
S/B	138	85	45	22	10	5	3	2	1
% Conversion	71	43	23	11	5	2	1	0	0

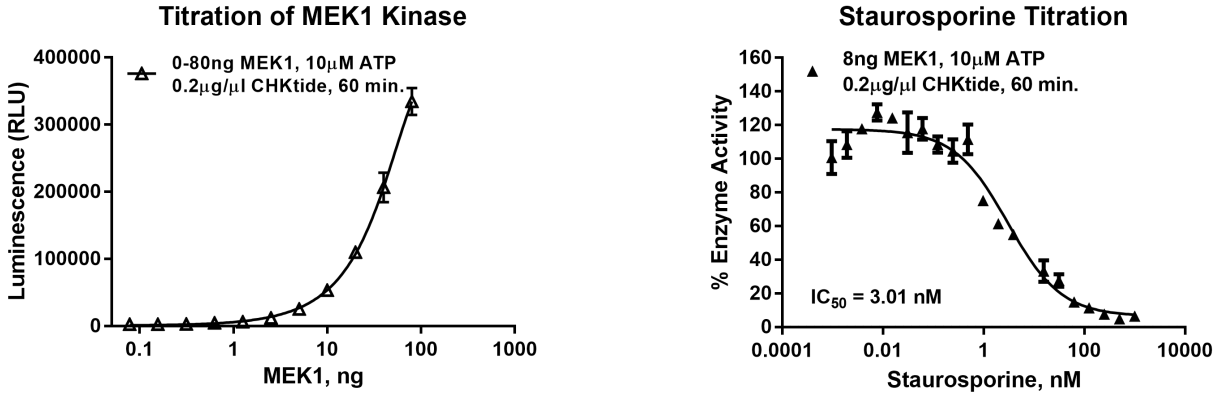

Figure 3. MEK1 Kinase Assay Development. (A) MEK1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 8ng of MEK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MEK1 Kinase Enzyme System	10µg	VA7216
	1mg	VA7217
ADP-Glo™ + MEK1 Kinase Enzyme System	1 Each	VA7218