ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

MEKK3 Kinase Assay

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Scientific Background:

MEKK3 is a member of the MEKK family of protein kinases that directly regulate SAPK and ERK pathway but not the p38 pathway. MEKK3 modulates SAPK and ERK activity by activating the upstream targets SEK and MEK1/2, respectively (1). KSR2 can regulate the activity of MEKK3 and can reduce MEKK3-mediated ERK activation. Knockout of the MEKK3 gene in mice leads to disruption of blood vessel development and the blockage of angiogenesis in these mice is not a result of decreased VEGF1 expression but rather due to an intrinsic defect in Mekk3 -/- endothelial cells (2). Thus, MEKK3 is necessary for blood vessel development and may be a possible target for drugs that control angiogenesis.

1. Ellinger-Ziegelbauer, H. Et al: Direct activation of the stress-activated protein kinase (SAPK) and extracellular signal-regulated protein kinase (ERK) pathways by an inducible mitogen-activated protein kinase/ERK kinase kinase 3 (MEKK) derivative. J. Biol. Chem. 272: 2668-2674, 1997.

2. Yang, J. et al: Mekk3 is essential for early embryonic cardiovascular development. Nature Genet. 24: 309-313, 2000.

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.

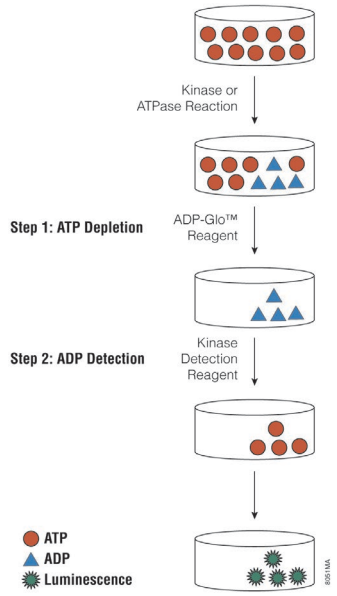

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

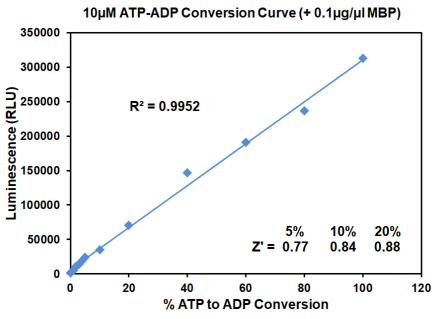

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	9.38	0
Luminescence	202,362	97,116	70,034	46,613	20,281	10,157	3,420
S/B	59	28	20	14	6	3	1
% Conversion	90	42	29	19	6	2	0

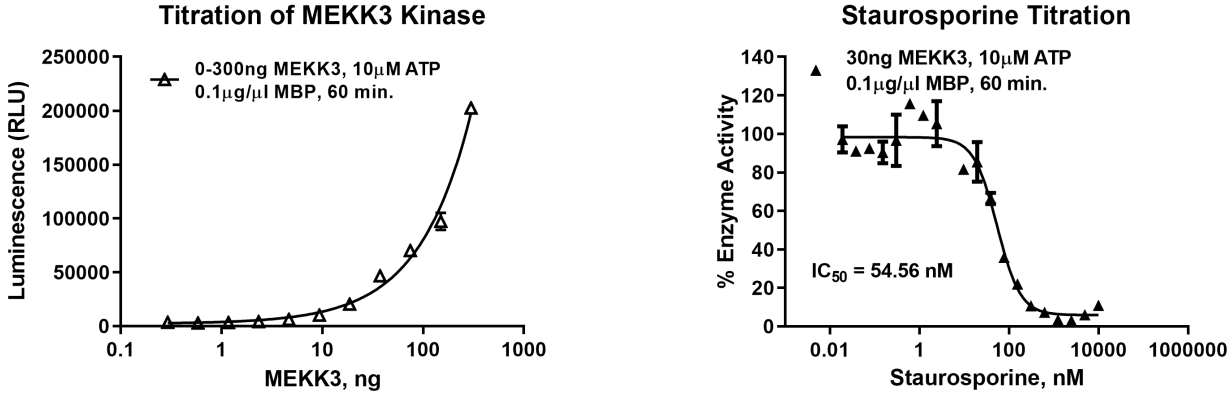

Figure 3. MEKK3 Kinase Assay Development. (A) MEKK3 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 30ng of MEKK3 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MEKK3 Kinase Enzyme System	10µg	VA7498
	1mg	VA7499
ADP-Glo™ + MEKK3 Kinase Enzyme System	1 Each	VA7500