ADP-Glo™ Kinase Assay Application Note

Dual-Specificity Kinase Series

MEK5 Kinase Assay

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Scientific Background:

MEK5 or MAP2K5 (mitogen-activated protein kinase kinase 5) is a dual specificity protein kinase that belongs to the MAP kinase kinase family which specifically interacts with and activates MAPK7/ERK5. MEK5 itself can be phosphorylated and activated by MAP3K3/MEKK3, as well as by atypical protein kinase C isoforms (aPKCs). The signal cascade mediated by MEK5is involved in growth factor stimulated cell proliferation and muscle cell differentiation (1). The rs3743354 polymorphism in the MEK5 promoter may affect the risk of developing colorectal cancer (CRC) (2).

1. Zhou, G..et.al: Components of a new human protein kinase signal transduction pathway. J. Biol. Chem. 270: 12665-12669, 1995.

2. Diao.D. et.al: Mitogen/extracellular signal-regulated kinase kinase-5 promoter region polymorphisms affect the risk of sporadic colorectal cancer in a southern Chinese population. DNA Cell Biol. 2012 Mar;31(3):342-9. doi: 10.1089/dna.2011.1232. Epub 2011 Aug 23.

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.

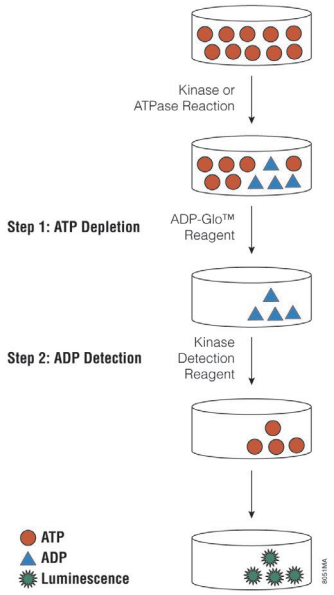

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

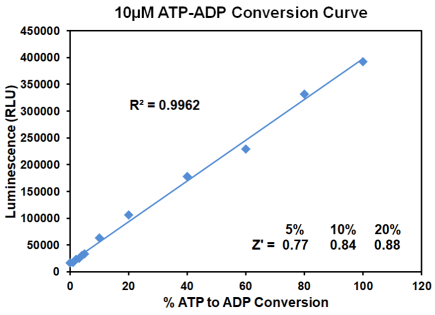

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	4.69	2.34	1.17	0
Luminescence	344,899	336,033	330,880	307,396	251,750	167,270	95,489	56,113	39,605	22,058
S/B	16	15	15	14	11	8	4	3	2	1
% Conversion	86	84	82	76	62	39	20	10	6	0

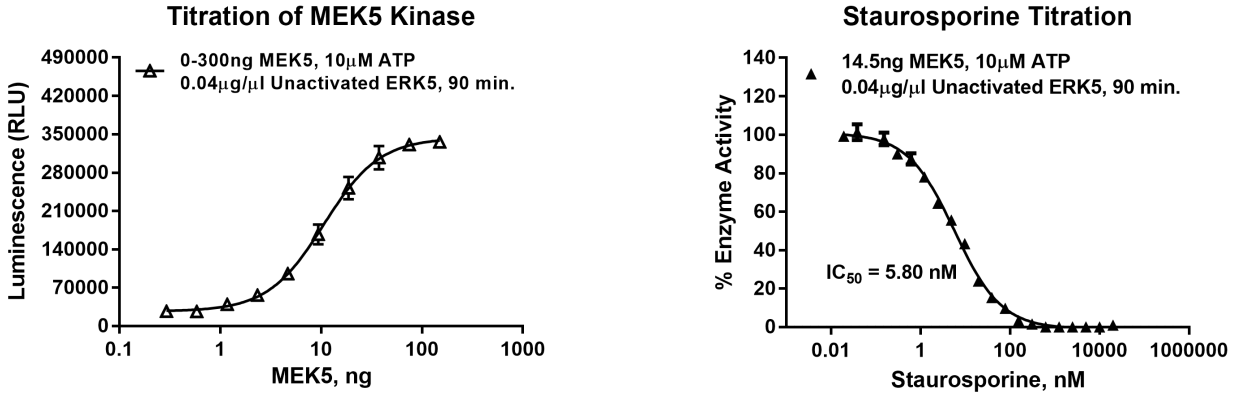

Figure 3. MEK5 Kinase Assay Development. (A) MEK5 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 14.5ng of MEK5 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MEK5 Kinase Enzyme System	10µg	VA7222
	1mg	VA7223
ADP-Glo™ + MEK5 Kinase Enzyme System	1 Each	VA7224