ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

HGK Kinase Assay

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Scientific Background:

HGK is the mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4) and a member of the serine/threonine protein kinase family. HGK has been shown to specifically activate MAPK8/JNK (1). The activation of MAPK8 by HGK can be inhibited by dominant-negative mutants of MAP3K7/TAK1, MAP2K4/MKK4, and MAP2K7/MKK7, which suggest that this kinase functions through the MAP3K7-MAP2K4-MAP2K7 kinase cascade and mediates TNF-α signaling. HGK-dependent signaling inhibits PPAR□-responsive gene expression, adipogenesis, and insulin-stimulated glucose transport (2).

1. Yao, Z. et.al: A novel human STE20-related protein kinase, HGK,that specifically activates the c-jun N-terminal kinase signaling pathway. J. Biol. Chem. 274: 2118-2125, 1999.

2. Tang, X. et.al: An RNA interference-based screen identifies MAP4K4/NIK as a negative regulator of PPAR-gamma, adipogenesis, and insulin-responsive hexose transport. Proc. Nat. Acad. Sci. 103: 2087-2092, 2006.

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.

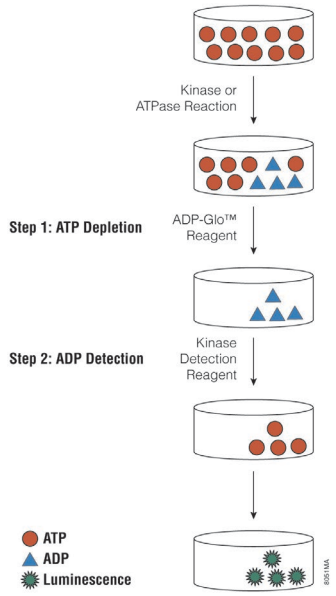

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

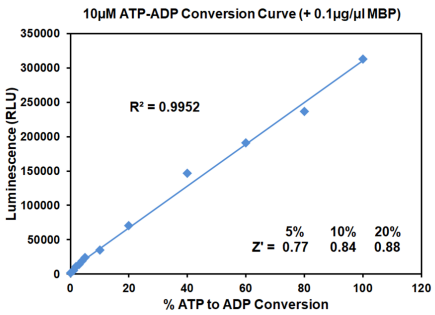

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	1.88	0.94	0.47	0
Luminescence	157,447	132,617	122,435	98,264	76,107	56,072	37,720	21,989	14,227	8,174	2,962
S/B	53	45	41	33	26	19	13	7	5	3	1
% Conversion	69	58	53	42	32	23	14	7	4	1	0

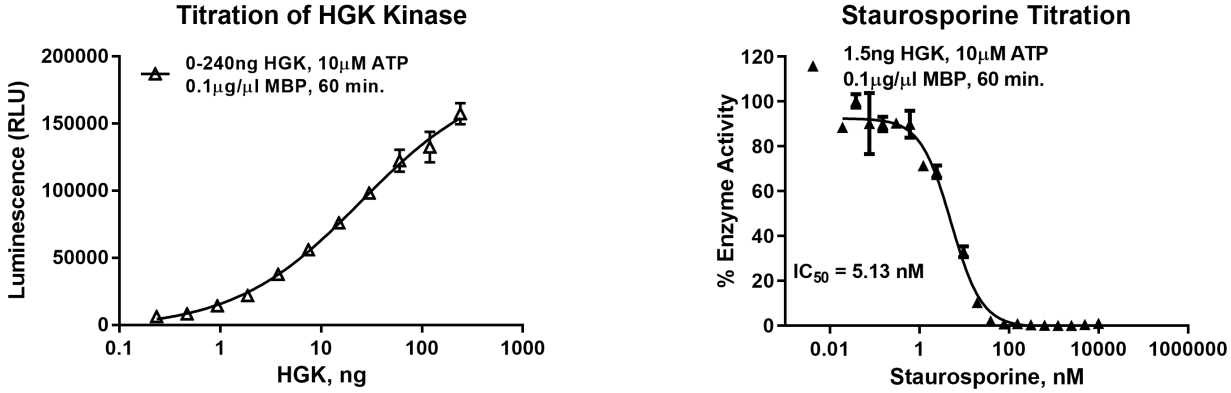

Figure 3. HGK Kinase Assay Development. (A) HGK enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1.5ng of HGK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
HGK Kinase Enzyme System	10µg	VA7471
	1mg	VA7472
ADP-Glo™ + HGK Kinase Enzyme System	1 Each	VA7473