ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

MARK3 Kinase Assay

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Scientific Background:

Microtubule affinity-regulating kinase 3 (MARK3) is a member of the PAR-1/MARK kinase family which play critical roles in polarity and cell cycle control and are regulated by 14-3-3 scaffolding proteins, as well as the LKB1 tumor suppressor kinase and atypical protein kinase C (PKC)(1). MARK3 is a dual-specificity protein kinase that controls entry into mitosis by dephosphorylating CDC2. MARK3 seems to be a positive regulator of the beta-catenin pathway and an inhibitor of the JNK pathway. MARK3, a regulator of polarity, is also a modulator of Wnt-beta-catenin signaling, indicating a link between two important developmental pathways (2).

1. Göransson, O. et al: Regulation of the polarity kinases PAR-1/MARK by 14-3-3 interaction and Phosphorylation. J. Cell Sci. 2006;119(19):4059-70.

2. Kato, T. et al: Isolation of a novel human gene, MARKL1, homologous to MARK3 and its involvement in hepatocellular carcinogenesis. Neoplasia. 2001;3(1):4-9.

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.

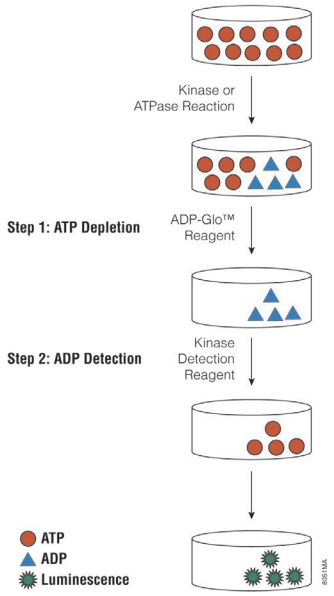

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

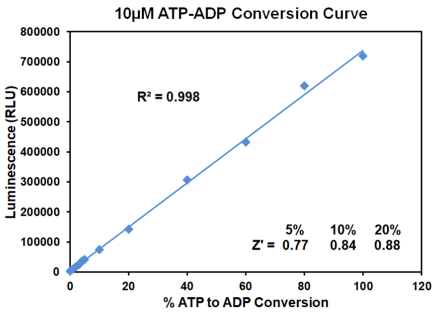

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	0.31	0.16	0
Luminescence	276,907	273,824	248,334	196,759	134,142	79,528	48,489	24,779	18,024	10,887	4,525
S/B	61	61	55	43	30	18	11	5	4	2	1
% Conversion	64	63	57	45	31	18	11	5	4	2	0

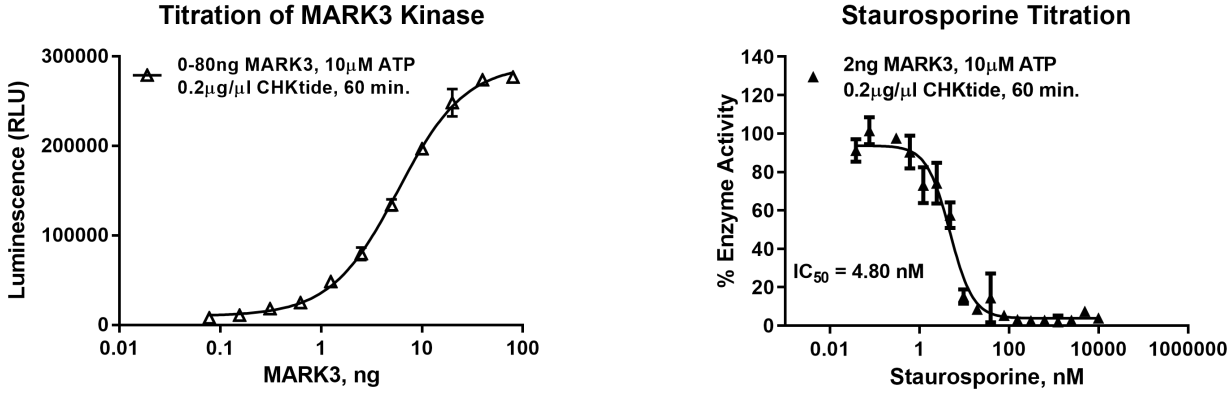

Figure 3. MARK3 Kinase Assay Development. (A) MARK3 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of MARK3 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MARK3 Kinase Enzyme System	10µg	VA7489
	1mg	VA7490
ADP-Glo™ + MARK3 Kinase Enzyme System	1 Each	VA7491