ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

MARK2 Kinase Assay

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

Scientific Background:

MARK2 or microtubule affinity-regulating kinase 2 is a member of the Par-1 family of serine/threonine protein kinases. MARK2 is an important regulator of cell polarity in epithelial and neuronal cells and controls the stability of microtubules through phosphorylation and inactivation of several microtubule-associating proteins (1). MARK2 is a key target of H. pylori CagA in the disorganization of gastric epithelial architecture underlying mucosal damage, inflammation, and carcinogenesis (2). MARK2 phosphorylates kinesin-like motor protein GAKIN/KIF13B to regulate axon formation. MARK2 function in the establishment of T cell polarity following engagement to an APC.

1. Yoshimura, Y. et.al: Polarity-regulating kinase partitioning-defective 1/microtubule affinity-regulating kinase 2 negatively regulates development of dendrites on hippocampal neurons. . J. Neurosci. 27:13098-13107, 2010

2. Saadat, I. et.al: Helicobacter pylori CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature 447: 330-333, 2007.

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.

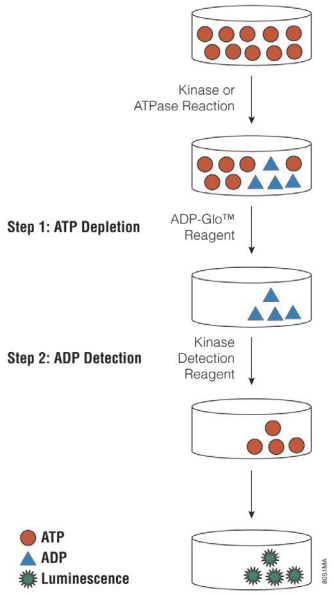

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

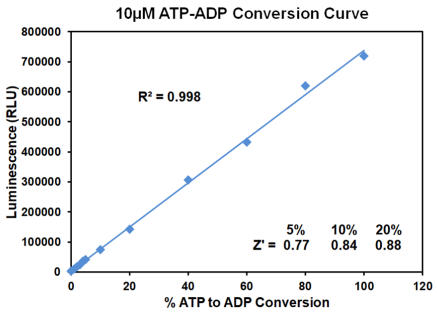

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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

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	60	30	15	7.50	3.75	1.88	0.94	0.47	0.23	0.12	0.06	0
Luminescence	364,876	351,318	269,999	156,460	106,500	53,591	32,294	16,577	10,002	5,781	4,098	1,962
S/B	186	179	138	80	54	27	16	8	5	3	2	1
% Conversion	72	70	54	31	21	11	7	4	2	1	1	0

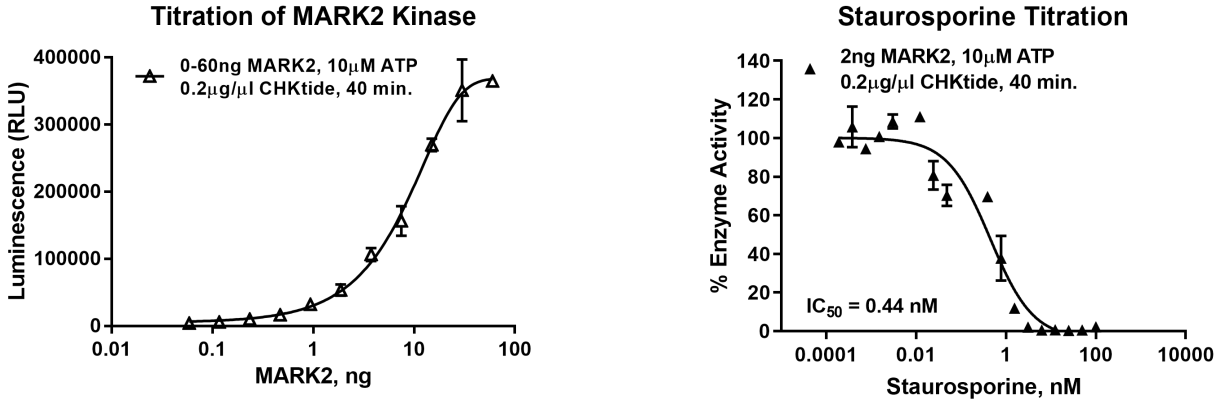

Figure 3. MARK2 Kinase Assay Development. (A) MARK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of MARK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MARK2 Kinase Enzyme System	10µg	VA7213
	1mg	VA7214
ADP-Glo™ + MARK2 Kinase Enzyme System	1 Each	VA7215