ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

MARK4 Kinase Assay

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Scientific Background:

MARK4 or microtubule affinity-regulating kinase 4 is a member of the Par-1 family of serine/threonine protein kinases. MARK4 is predominantly expressed in the brain and readily phosphorylates Tau, MAP2 and MAP4. MARK4 co-localizes with the centrosome and microtubules in cultured cells. Overexpression of MARK4 causes thinning out of the microtubule network concomitant with the reorganization of microtubules into bundles (1). MARK4 provides a growth advantage to cells and the up-regulation of this kinase during focal ischaemia may represent an interesting new target for pharmacological intervention. MARK4 is expressed during the cell cycle and is linked to aberrant centrosomes in glioma cells (2).

1. Trinczek, B. et al: MARK4 is a novel microtubule-associated proteins/microtubule affinity-regulating kinase that binds to the cellular microtubule network and to centrosomes. J Biol Chem. 2004 Feb 13;279(7):5915-23.

2. Magnani, I. et.al: Multiple localization of endogenous MARK4L protein in human glioma. Cell Oncol, 2009. PMID 19759416.

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.

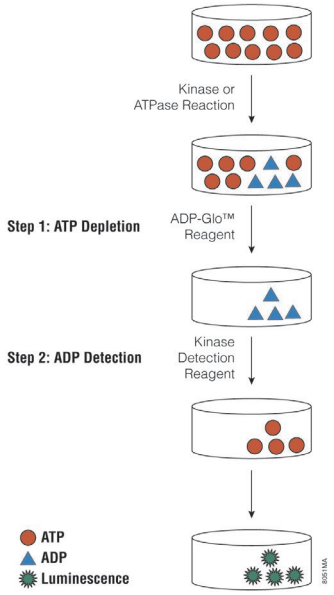

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

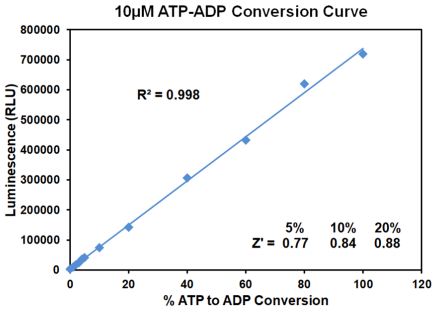

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	120	60	30	15	7.50	3.75	1.88	0.94	0.47	0.23	0
Luminescence	205,067	192,831	183,440	151,499	103,176	61,877	33,359	17,331	9,403	5,123	1,577
S/B	130	122	116	96	65	39	21	11	6	3	1
% Conversion	73	69	65	53	36	21	10	5	2	0	0

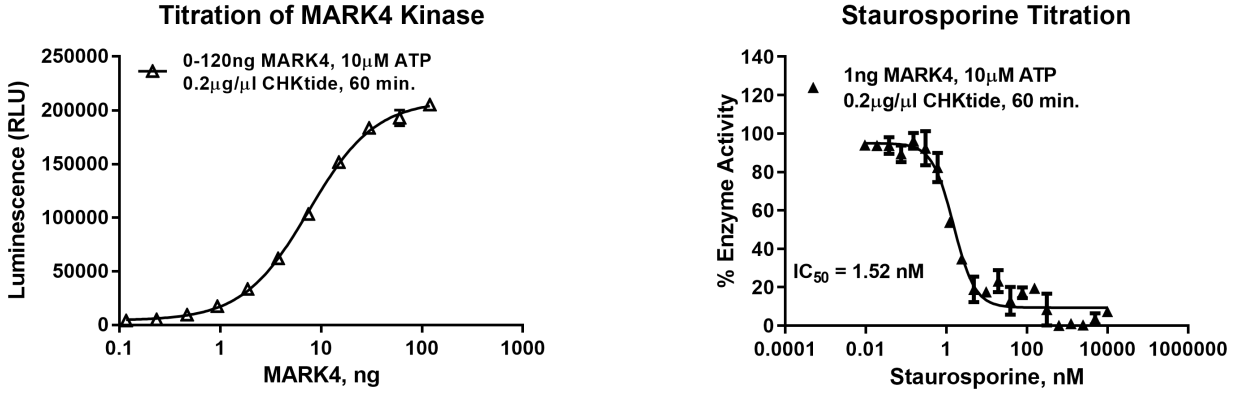

Figure 3. MARK4 Kinase Assay Development. (A) MARK4 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of MARK4 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MARK4 Kinase Enzyme System	10µg	VA7492
	1mg	VA7493
ADP-Glo™ + MARK4 Kinase Enzyme System	1 Each	VA7494