ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

DCAMKL1 Kinase Assay

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Scientific Background:

DCAMKL1 or doublecortin-like kinase 1 contains two N-terminal doublecortin domains (which bind microtubules and regulate microtubule polymerization), a C-terminal serine/threonine protein kinase domain (which shows substantial homology to Ca2+/calmodulin-dependent protein kinase), and a serine/proline-rich domain in between the doublecortin and the protein kinase domains (which mediates multiple protein-protein interactions) (1). DCAMKL1 is a microtubule-associated kinase that can undergo autophosphorylation. DCAMKL1 has microtubule-polymerizing activity that is independent of its protein kinase activity (2). DCAMKL1 is involved in several different cellular processes, including neuronal migration, retrograde transport, neuronal apoptosis and neurogenesis.

1. Ohmae, S. et.al: Molecular identification and characteri-zation of a family of kinases with homology to Ca(2+) /calmodulin-dependent protein kinases I/IV. J. Biol. Chem. 281: 20427-20439, 2006.
2. Lin, P. T. et,al: DCAMKL1 encodes a protein kinase with homology to doublecortin that regulates microtubule polymerization. J. Neurosci. 20: 9152-9161, 2000.

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.

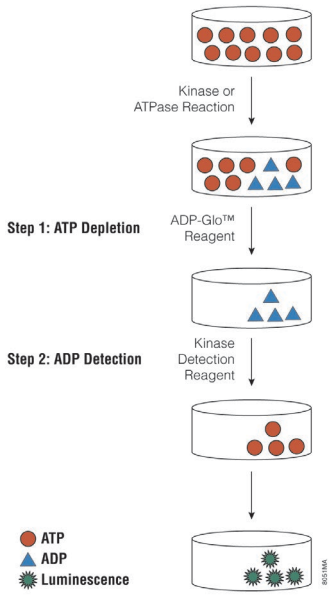

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

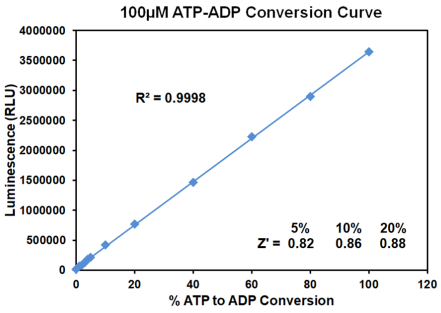

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	0
Luminescence	1,125,980	352,917	135,300	66,687	47,263	20,303	12,431	7,461
S/B	151	47	18	9	6	3	2	1
% Conversion	32	10	4	2	1	0	0	0

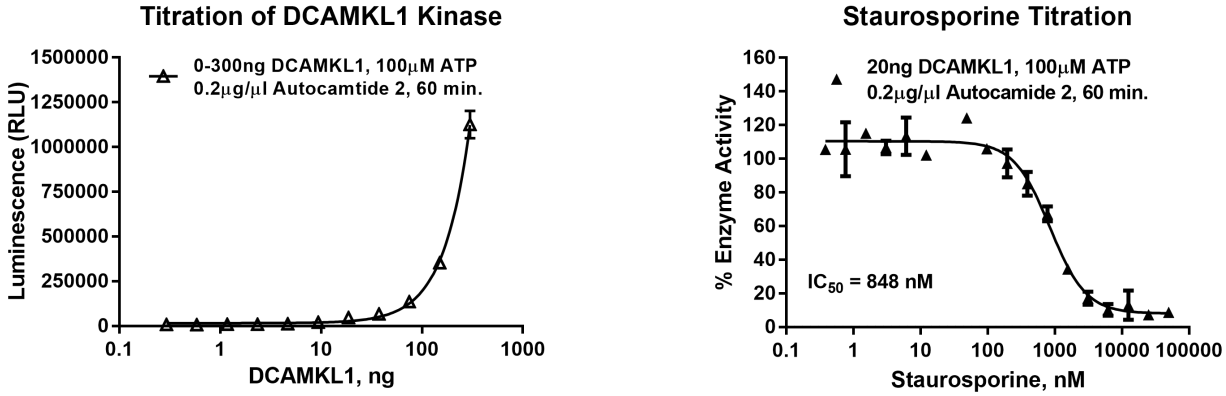

Figure 3. DCAMKL1 Kinase Assay Development. (A) DCAMKL1 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of DCAMKL1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DCAMKL1 Kinase Enzyme System	10µg	VA7075
	1mg	VA7076
ADP-Glo™ + DCAMKL1 Kinase Enzyme System	1 Each	VA7077