ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

DAPK2 Kinase Assay

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Scientific Background:

DAPK2 or death-associated protein kinase 2 belongs to a family of proapoptotic Ca(2+)/calmodulin-regulated serine/threonine kinases. Overexpression of DAPK2 induces cell apoptosis. DAPK2 has been shown to be a novel Sp1-dependent target gene for E2F1 and Krüppel-like factor 6 (KLF6) in cell death response (1). Both E2F1 and KLF6 strongly activate the DAPK2 promoter. DAPK2 plays a role in granulopoiesis where it is highly expressed. β-catenin can block anoikis of malignant kidney and intestinal epithelial cells and promote their anchorage-independent growth by down-regulating DAPK2 (2). β-catenin-induced down-regulation of DAPK2 requires the presence of the transcription factor TCF-4.

1. Britschgi A. et al: DAPK2 is a novel E2F1/KLF6 target gene involved in their proapoptotic function. Oncogene. 2008 Sep 25;27(43):5706-16.

2. Li, H. et al: Down-regulation of death-associated protein kinase-2 is required for beta-catenin-induced anoikis resistance of malignant epithelial cells. J Biol Chem. 2009 Jan 23;284(4):2012-22.

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.

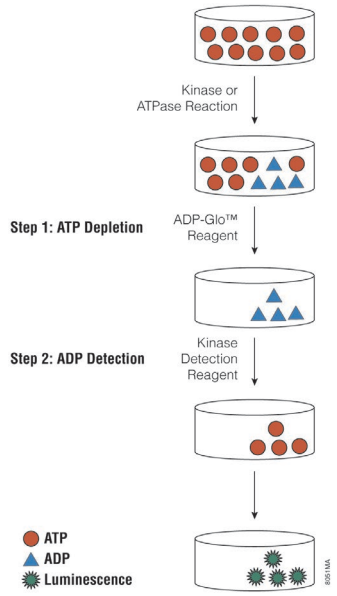

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

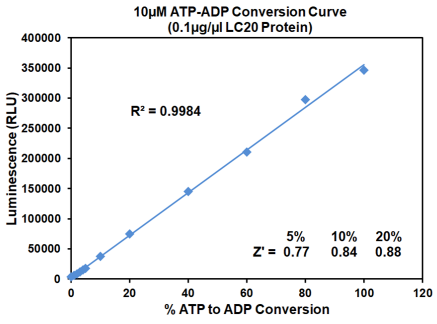

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0
Luminescence	193,570	104,031	88,257	60,119	51,359	31,767	22,716	14,587	8,583	4,448
S/B	44	23	20	14	12	7	5	3	2	1
% Conversion	54	29	24	16	14	8	6	4	2	0

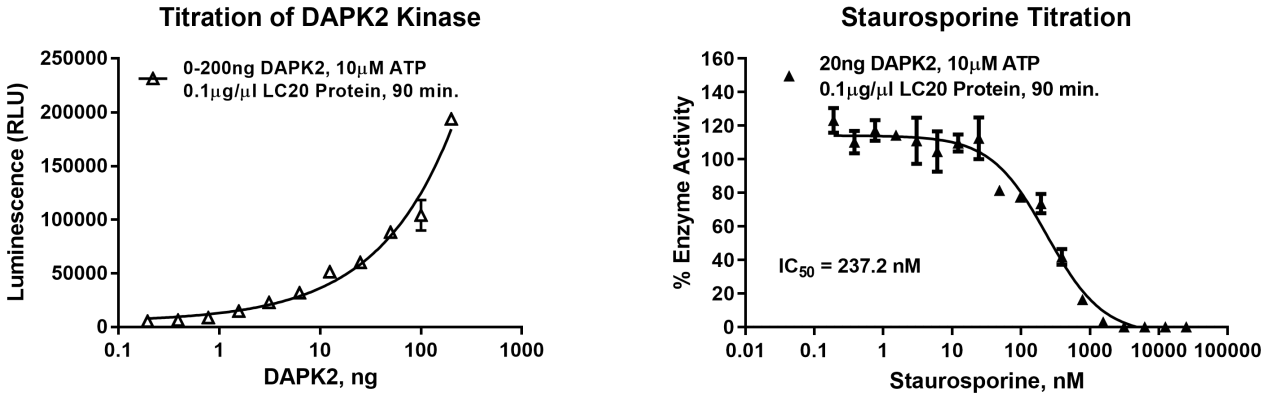

Figure 3. DAPK2 Kinase Assay Development. (A) DAPK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of DAPK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DAPK2 Kinase Enzyme System	10µg	VA7417
	1mg	VA7418
ADP-Glo™ + DAPK2 Kinase Enzyme System	1 Each	VA7419