ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

DAPK3 Kinase Assay

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Scientific Background:

DAPK3 or Death-associated protein kinase 3 (also known as ZIP) plays a role in apoptosis (1). DAPK3 is a nuclear serine/threonine-specific kinase that phosphorylates core histones H3 and H4, and myosine light chain in vitro. DAPK3 interacts with transcription and splicing factors as well as with pro-apoptotic protein Par-4 suggesting that it participates in multiple cellular processes. DAPK3 contains a leucine zipper structure at its C terminus and this region is responsible for binding to ATF4. The leucine zipper domain is necessary for the homodimerization of DAPK3 as well as for the activation of the kinase (2).

1. Kawai, T. et al: ZIP kinase, a novel serine/threonine kinase which mediates apoptosis.Mol. Cell Biol. 1998;18(3):1642-51.

2. Preuss, U. et al. Novel mitosis-specific phosphorylation of histone H3 at Thr11 mediated by Dlk/ZIP kinase.Nucleic Acids Res. 2003; 31(3):878-85.

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.

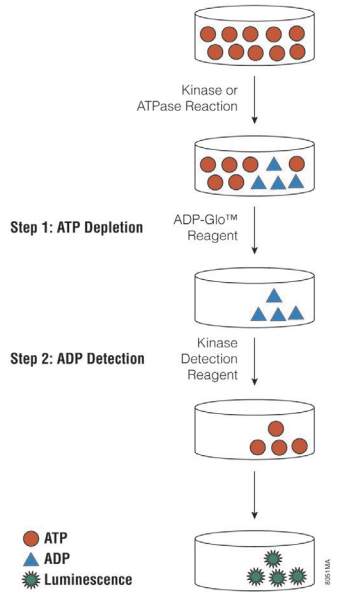

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

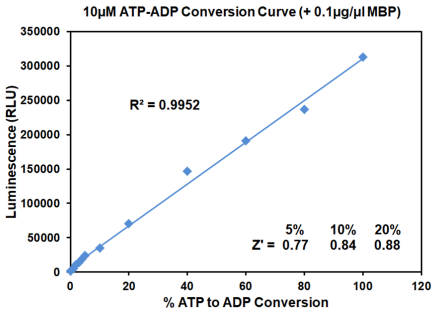

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0.29	0
Luminescence	212,880	165,225	167,261	125,564	86,482	43,427	23,682	11,357	6,226	3,162	1,895	839
S/B	254	197	199	150	103	52	28	14	7	4	2	1
% Conversion	72	55	56	42	28	13	6	2	0	0	0	0

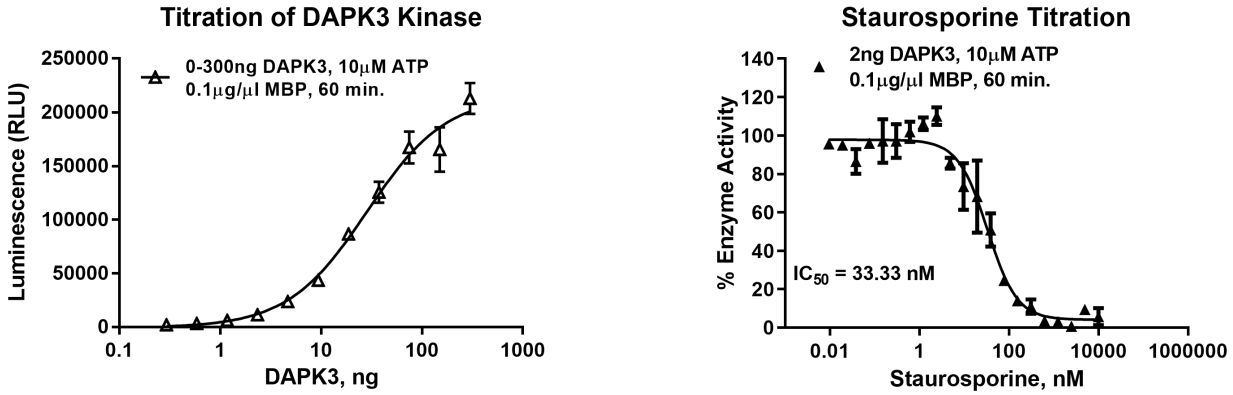

Figure 3. DAPK3 Kinase Assay Development. (A) DAPK3 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of DAPK3 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DAPK3 Kinase Enzyme System	10µg	VA7420
	1mg	VA7421
ADP-Glo™ + DAPK3 Kinase Enzyme System	1 Each	VA7422