ADP-Glo™ Kinase Assay Application Note

Ser-Thr Kinase Series

PLK2 Kinase Assay

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Scientific Background:

PLK2 or Polo-like kinase 2 is a serum-inducible kinase that is a member of the Polo family of serine/threonine protein kinases which play an essential role in normal cell division (1). PLK2 phosphorylates the P4.1-associated protein (CPAP), a human homologue of SAS-4, in procentriole formation during the centrosome cycle (2). PLK2 may also play a role as a tumor suppressor since it interacts with TSC1 protein which in turn inhibits mTOR signaling, tumor growth and chemosensitivity under hypoxic conditions. Human lung tumor cells deficient in PLK2 grow larger than control tumors, and PLK2 interacts with endogenous TSC1 protein in these tumors.

1. Chang J, et al: PLK2 phosphorylation is critical for CPAP function in procentriole formation during the centrosome cycle. EMBO J, 2010 Jul 21. PMID 20531387.

2. Matthew EM, et al: The p53 target Plk2 interacts with TSC proteins impacting mTOR signaling, tumor growth and chemosensitivity under hypoxic conditions. Cell Cycle, 2009 Dec 15. PMID 20054236.

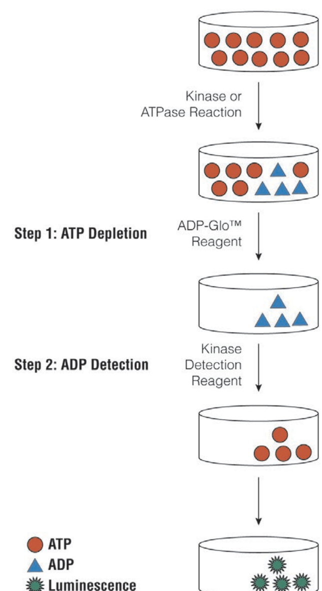

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

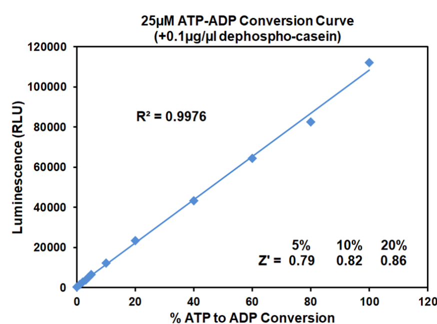

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	113	56.25	28.13	14.06	7.03	3.52	0
Luminescence	21,544	8,519	6,302	2,985	1,539	631	369
S/B	58	23	17	8	4	2	1
% Conversion	19	7	5	2	1	0	0

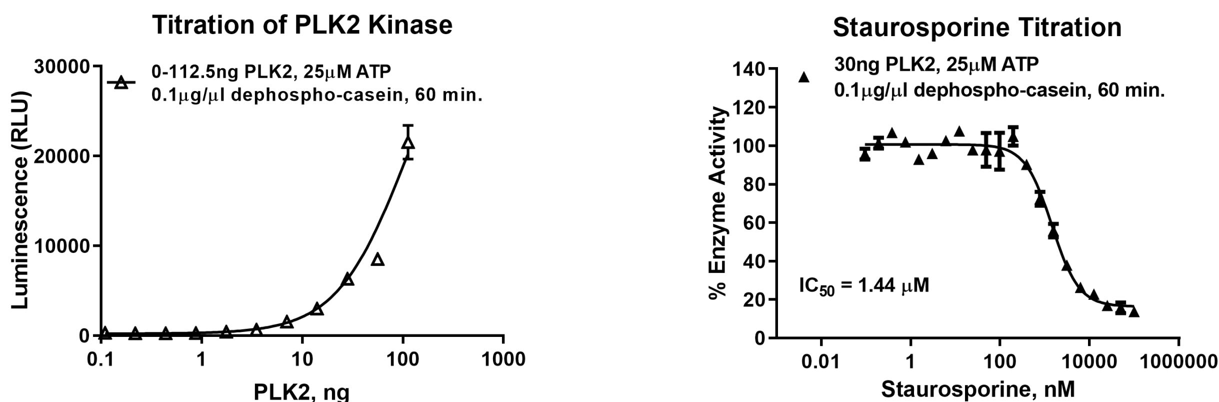

Figure 3. PLK2 Kinase Assay Development. (A) PLK2 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 30ng of PLK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PLK2 Kinase Enzyme System	10µg	VA7537
	1mg	VA7538
ADP-Glo™ + PLK2 Kinase Enzyme System	1 Each	VA7539