ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

PLK4 Kinase Assay

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Scientific Background:

PLK4 or polo-like kinase 4 is a member of the polo family of serine/threonine protein kinases which localizes to centrioles, complex microtubule-based structures found in centrosomes, and regulates centriole duplication during the cell cycle. The overexpression of PLK4 triggered the simultaneous formation of multiple procentrioles around each preexisting centriole which resulting in centriole amplification and thus, Plk4-induced centriole biogenesis in human cells (1).The reduced Plk4 gene dosage increases the probability of mitotic errors and cancer development (2).

1. Kleylein-Sohn, J. et.al: Plk4-induced centriole biogenesis in human cells. Dev. Cell 13: 190-202, 2007.

2. Ko, M. A. et.al: Plk4 haploinsufficiency causes mitotic infidelity and carcinogenesis. Nature Genet. 37: 883-888, 2005.

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.

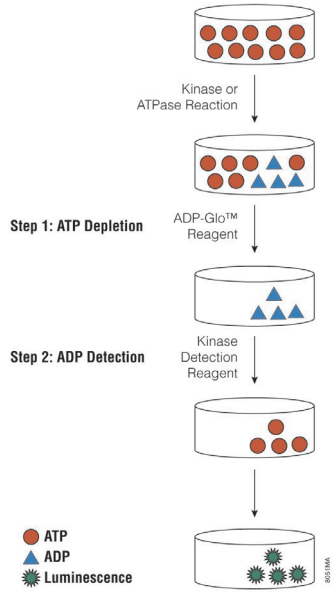

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

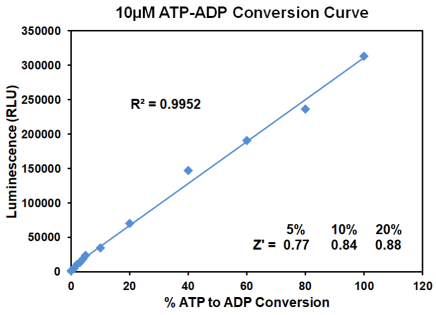

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	0
Luminescence	138,147	64,467	31,355	14,932	6,070	3,047	1,273
S/B	109	51	25	12	5	2	1
% Conversion	65	29	14	6	1	0	0

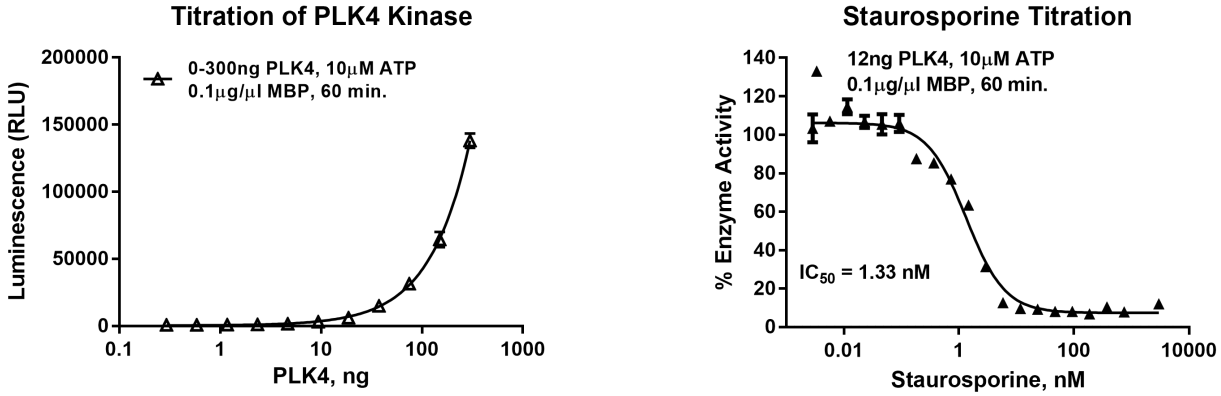

Figure 3. PLK4 Kinase Assay Development. (A) PLK4 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 12ng of PLK4 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PLK4 Kinase Enzyme System	10µg	VA7258
	1mg	VA7259
ADP-Glo™ + PLK4 Kinase Enzyme System	1 Each	VA7260