ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

AURORA C Kinase Assay

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Scientific Background:

AURORA C is a member of mitotic serine/threonine kinases that regulate centrosome maturation, chromosome segregation, and cytokinesis. AURORA C has been shown to contribute to oncogenic transformation and is frequently overexpressed and amplified in many human tumors (1). AURORA C overexpression has been correlated with centrosome amplification, which can be a driving cause of genomic instability in tumor cells. AURORA C is an important regulator of both genomic integrity and cell cycle progression in cancer cells and is an attractive target for anticancer drug development (2).

1. Steven, L. et al: Targeting Aurora-2 Kinase in Cancer. Molecular Cancer Therapeutics. 2003;2: 589-595

2. Shingo, T. et al: The centrosomal protein Lats2 is a phosphorylation target of Aurora-A kinase. Genes to Cells. 2004; 9: 383-397

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.

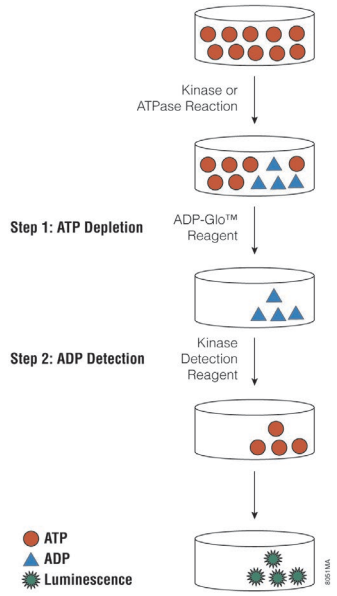

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

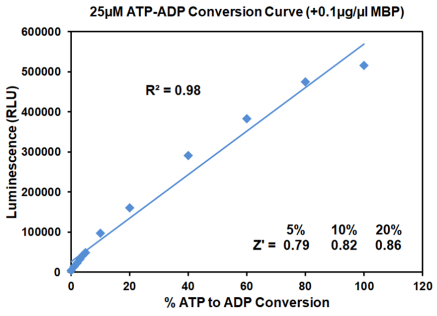

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	1.88	0
Luminescence	365,552	249,435	159,931	70,172	27,861	11,363	5,692	3,355	2,195
S/B	167	114	73	32	13	5	3	2	1
% Conversion	64	42	26	9	1	0	0	0	0

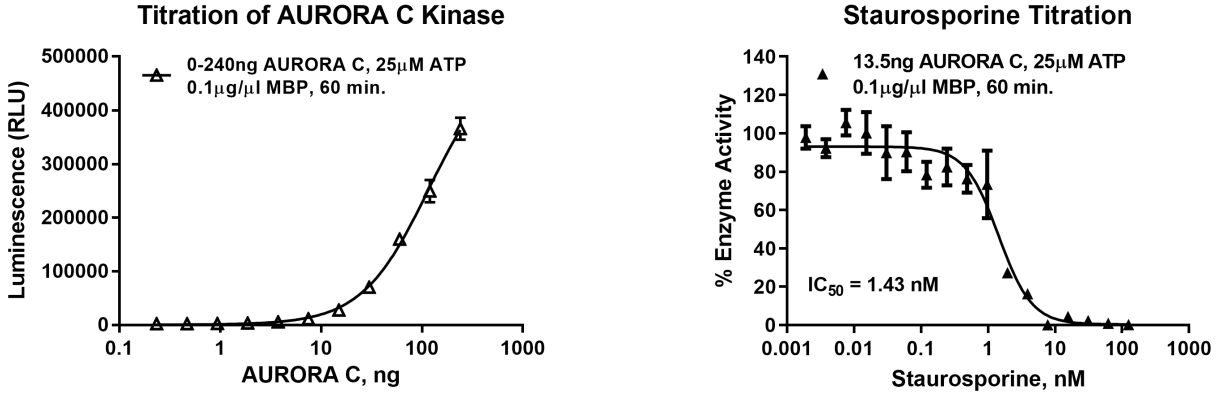

Figure 3. AURORA C Kinase Assay Development. (A) AURORA C enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 13.5ng of AURORA C to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
AURORA C Kinase Enzyme System	10µg	VA7381
	1mg	VA7382
ADP-Glo™ + AURORA C Kinase Enzyme System	1 Each	VA7383