Aurora B Kinase Assay

By Hicham Zegzouti, Ph.D., Jolanta Vidugiriene, Ph.D., Kevin Hsiao, M.S., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

AURORA B is a member of the Aurora kinase family that associates with microtubules during chromosome movement and segregation. AURORA B localizes to the microtubules near kinetochores, specifically to the specialized microtubules called K-fibers (1). AURORA B inhibits the microtubule depolymerizing activity of mitotic centromere-associated kinesin (MCAK) by phosphorylating MCAK on Ser92 (2). This phosphorylation also regulates MCAK translocalization from kinetochores to the centromere. AURORA B has been identified as a target for the development of new anticancer agents since inhibition of AURORA B gives rise to the more pronounced antiproliferative phenotype.

1. Shannon, K B. et al: Chromosome dynamics: new light on Aurora B kinase function. Curr Biol. 2002 Jul 9;12(13):R458-60.
2. Andrews, P. D. et al: Aurora B regulates MCAK at the mitotic centromere. Dev. Cell 6: 253-268, 2004.

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.

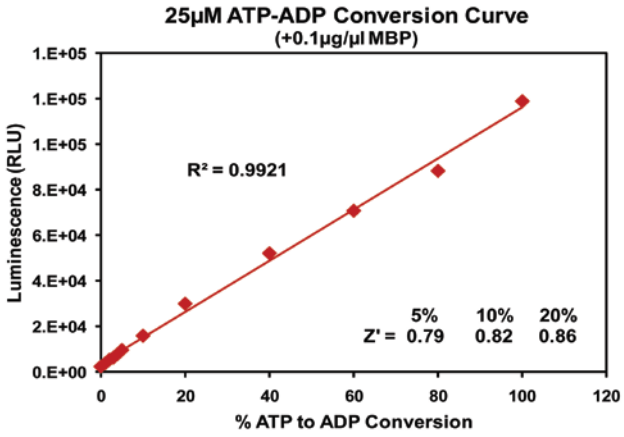

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase 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 60 minutes.
- 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 0.5-1second).

Table 1. Aurora B 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.

Aurora B, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0
RLU	88163	76838	51388	34698	19608	10142	4801	2383	1874
S/B	47.0	41.0	27.4	18.5	10.5	5.4	2.6	1.3	1
% Conversion	76.6	66.3	43.1	27.9	14.1	5.4	1.8	0.8	0

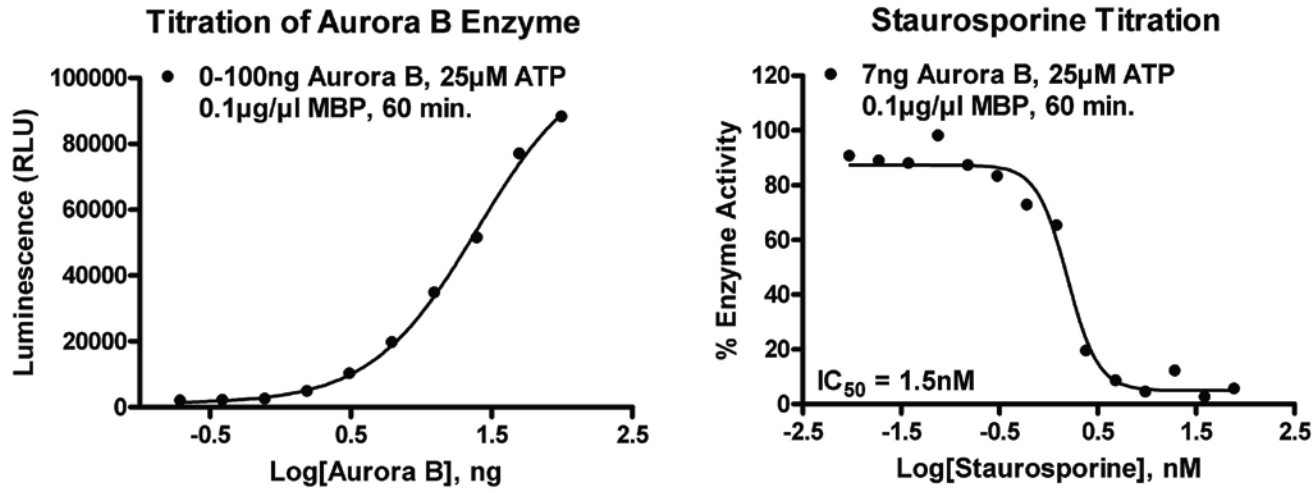

Figure 3. Aurora B Kinase Assay Development. (A) Aurora B enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 7ng of Aurora B to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
Aurora B Kinase Enzyme System	Promega	V3971
ADP-Glo™ + Aurora B Kinase Enzyme System	Promega	V9181

Aurora B Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.