

# Aurora A Kinase Assay

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## Scientific Background:

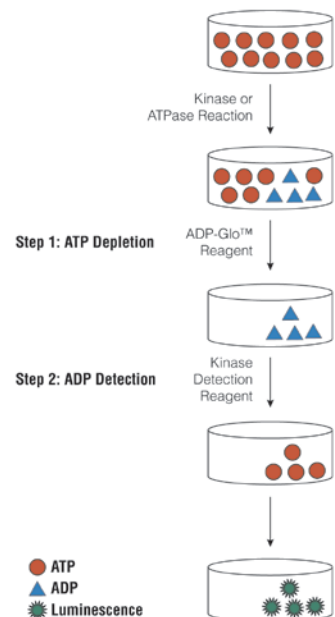
AURORA A belongs to a multigenic family of mitotic serine/threonine kinases which are involved in the control of chromosome segregation. AURORA A is involved in centrosome separation, duplication and maturation as well as in bipolar spindle assembly and stability (1). AURORA A is expressed and active at the highest level during G2-M phase of the cell cycle. Overexpression of AURORA A has been found to be correlated with the grade of various human solid tumours. Ectopic AURORA A overexpression in any culture cell line leads to polyploidy and centrosome amplification (2).

1. Dutertre, S. et al: On the role of aurora-A in centrosome function. *Oncogene*. 2002 Sep 9;21(40):6175-83.
2. Katayama, H. et al: The Aurora kinases: role in cell transformation and tumorigenesis. *Cancer Metastasis Rev*. 2003 Dec;22(4):451-64.

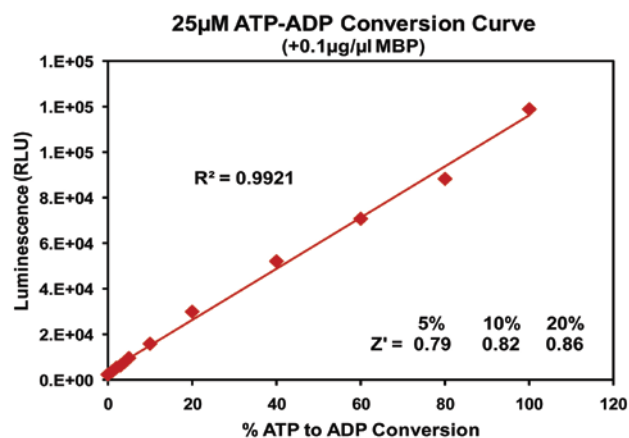
## ADP-Glo™ Kinase Assay

### 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 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.



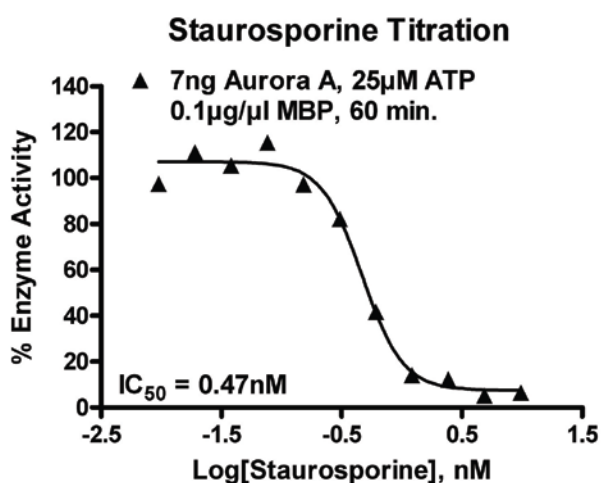
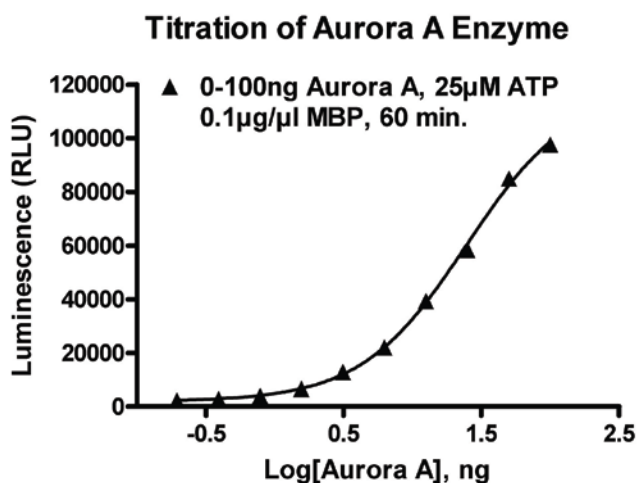
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html)

## Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1  $\mu$ l of inhibitor or (5% DMSO)
  - 2  $\mu$ l of enzyme (defined from table 1)
  - 2  $\mu$ l of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5  $\mu$ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10  $\mu$ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

**Table 1. Aurora A 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 A, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0
RLU	97688	84954	58324	39334	22065	12924	6612	4020	2306
S/B	42.4	36.8	25.3	17.1	9.6	5.6	2.9	1.7	1
% Conversion	85.3	73.7	49.4	32.1	16.3	8.0	2.2	1.8	0



**Figure 3. Aurora A Kinase Assay Development.** (A) Aurora A enzyme was titrated using 25 $\mu$ M 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 A to determine the potency of the inhibitor (IC<sub>50</sub>).

### Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
Aurora A Kinase Enzyme System	Promega	V1931
ADP-Glo™ + Aurora A Kinase Enzyme System	Promega	V9081

Aurora A Kinase Buffer: 40mM Tris,7.5; 20mM MgCl<sub>2</sub>; 0.1mg/ml BSA; 50 $\mu$ M DTT.