AXL Kinase Assay

By Jolanta Vidugiriene, Ph.D., Hicham Zegzouti, Ph.D., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

AXL is a member of the receptor tyrosine kinase family which has oncogenic potential and is implicated in human myeloid leukemia (1). AXL is a member of a complex signaling network that is involved in the control of cell proliferation and differentiation. Overexpression of AXL cDNA in NIH 3T3 cells induces neoplastic transformation of these cells with the concomitant appearance of a 140kDa AXL tyrosine-phosphorylated protein (2). Expression of AXL cDNA in the baculovirus system results in the expression of the appropriate recombinant protein that is recognized by antiphosphotyrosine antibodies, confirming that the AXL protein is tyrosine phosphorylated.

1. O'Bryan, J.P. et al: Axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. Mol Cell Biol. 1991 Oct;11(10):5016-31.

2. Janssen, J.W. et al:, A novel putative tyrosine kinase receptor with oncogenic potential. Oncogene. 1991 Nov;6(11):2113-20 .

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.

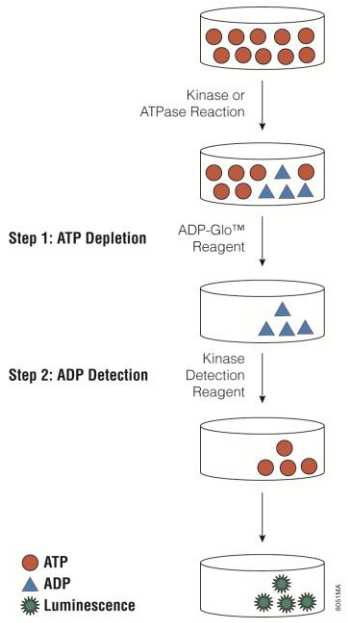

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

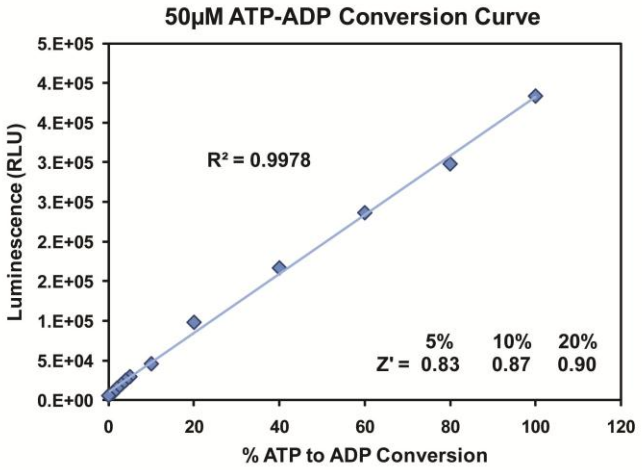

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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. AXL 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.

AXL, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
Luminescence	128782	118532	116678	112818	99176	90625	70374	50388	29904	10295	4624
S/B	27.9	25.6	25.2	24.4	21.5	19.6	15.2	10.9	6.5	2.2	1.0
% Conversion	50.8	46.5	45.7	44.0	38.3	34.7	26.1	17.7	9.0	0.7	0.0

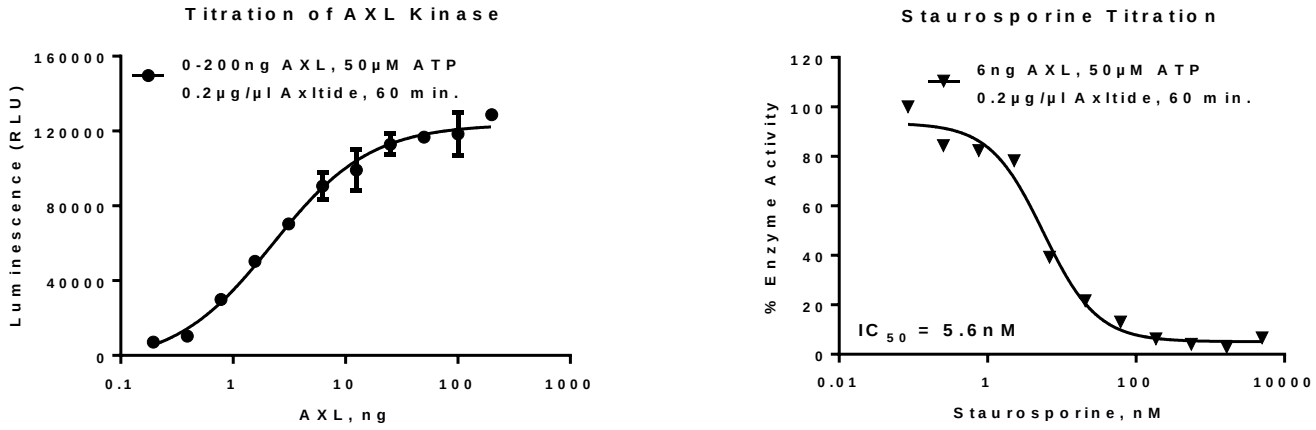

Figure 3. AXL Kinase Assay Development: (A) AXL enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 6ng of AXL to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
AXL Kinase Enzyme System	Promega	V3961
ADP-Glo + AXL Kinase Enzyme System	Promega	V9171

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

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
promega.com