ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

ABL2 Kinase Assay

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Scientific Background:

ABL2 (or ARG) is a nonreceptor cytoplasmic tyrosine kinase which is closely related to but distinct from ABL1. The similarity of ABL1 and ABL2 includes the tyrosine kinase domains and extends amino-terminal to include the SH2 and SH3 domains. ABL2 is involved in translocation with the ETV6 gene in human leukemia and has an altered expression in several human carcinomas (1). Two isoforms of ABL2 with different N-termini (1A and 1B) have been identified. The C-terminal domain of ABL2 contains two F-actin-binding sequences that perform a number of actions related to cell morphology and motility by interacting with actin filaments (2).

1. Barila, D. et al : An intramolecular SH3-domain interaction regulates c-Abl activity. Nature Genet. 18: 280-282, 1998.

2. Griesinger, F. et al: Identification of an ETV6-ABL2 fusion transcript in combination with an ETV6 point mutation in a T-cell acute lymphoblastic leukaemia cell line. Br J Haematol. 2002 Nov;119(2):454-8.

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.

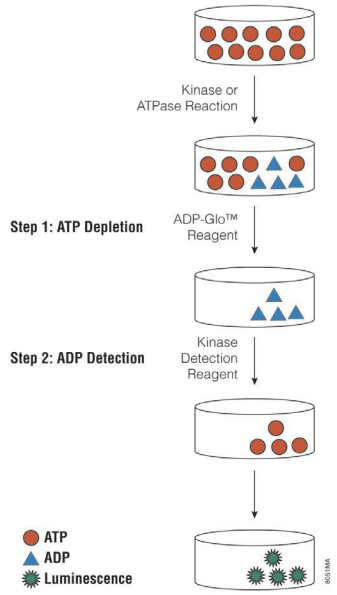

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

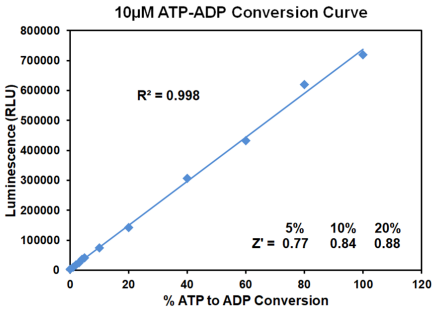

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	10	5	2.50	1.25	0.63	0.31	0.16	0.08	0.04	0.02	0
Luminescence	194,581	211,435	174,706	147,337	95,878	55,127	32,957	17,528	9,933	5,935	1,762
S/B	110	120	99	84	54	31	19	10	6	3	1
% Conversion	60	65	54	45	29	16	9	4	2	1	0

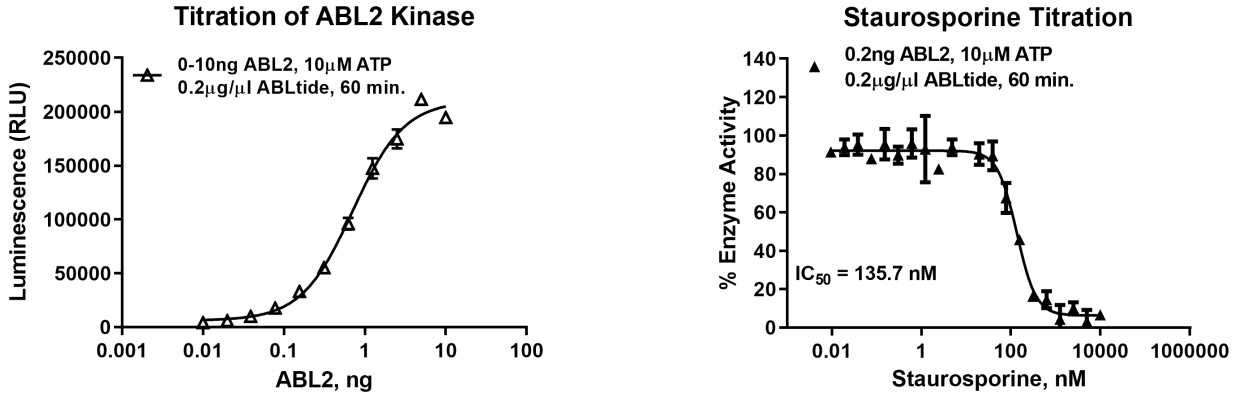

Figure 3. ABL2 Kinase Assay Development. (A) ABL2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 0.2ng of ABL2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
ABL2 Kinase Enzyme System	10µg	VA7369
	1mg	VA7370
ADP-Glo™ + ABL2 Kinase Enzyme System	1 Each	VA7371