

ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: ABL (Y253F)



ABL (Y253F) Kinase Assay

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Scientific Background:

ABL1 protooncogene encodes a cytoplasmic and nuclear protein tyrosine kinase that has been implicated in processes of cell differentiation, cell division, cell adhesion, and stress response. Activity of ABL protein is negatively regulated by its SH3 domain and deletion of the SH3 domain turns ABL1 into an oncogene (1). Translocation and head-to-tail fusion of the BCR and ABL1 genes is present in many cases of chronic myelogenous leukemia (2). The DNA-binding activity of the ubiquitously expressed ABL1 tyrosine kinase is regulated by CDK1-mediated phosphorylation, suggesting a cell cycle function for ABL1.

1. Barila, D. et al : An intramolecular SH3-domain interaction regulates c-Abl activity. *Nature Genet.* 18: 280-282, 1998.
2. Goldman, J M. et al : Targeting the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *New Eng. J. Med.* 344: 1084-1086, 2001.

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.

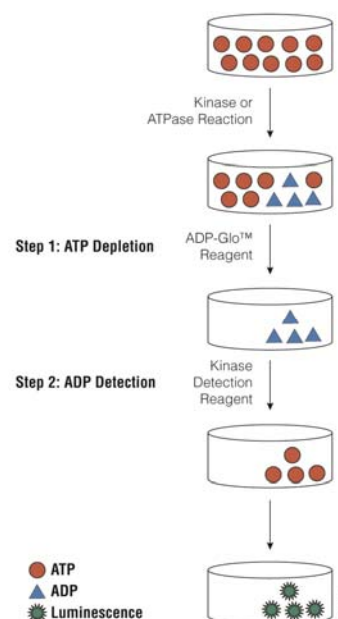


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

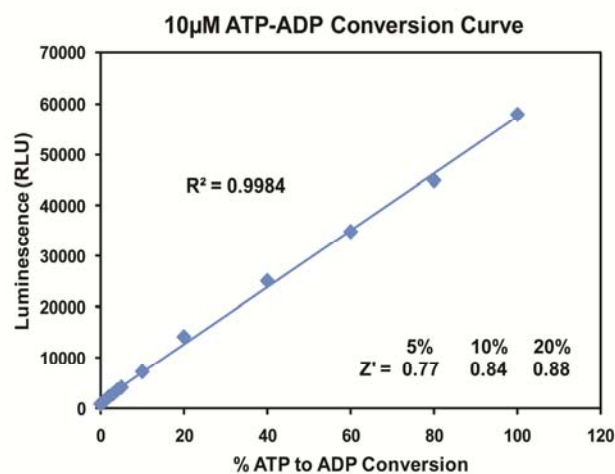
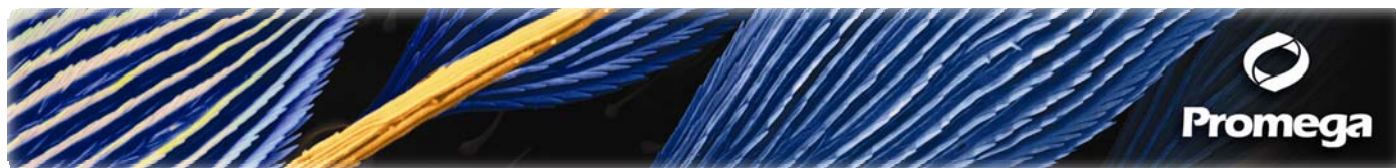


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, and the KES Protocol available at: <http://www.promega.com/tbs/tm313/tm313.html>, and <http://www.promega.com/KESProtocol>, respectively.

Protocol

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

Table 1. ABL (Y253F) 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.

ABL (Y253F), ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0
Luminescence	37908	37635	34891	31114	21697	12035	6229	3064	1499	146
S/B	260	258	239	213	149	82	43	21	10	1
% Conversion	46	45	43	39	24	13	7	3	1	0

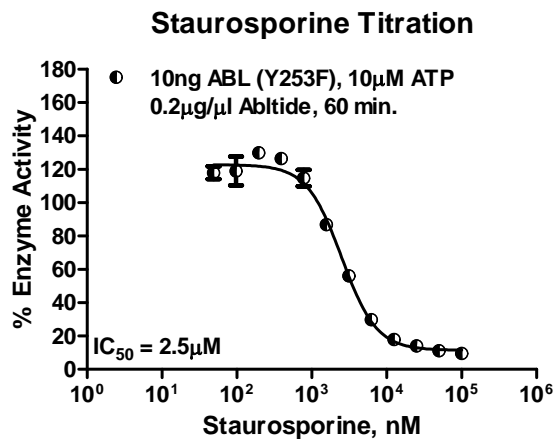
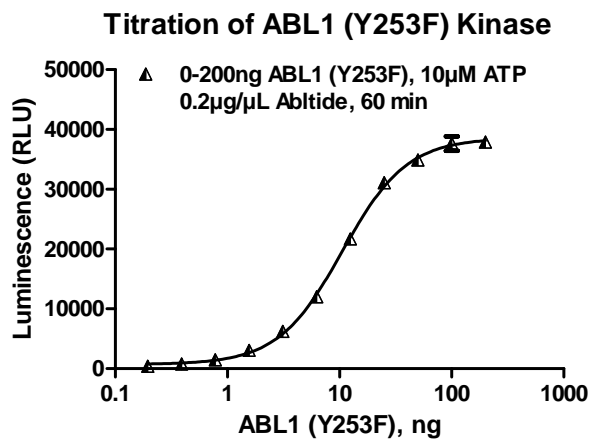


Figure 3. ABL (Y253F) Kinase Assay Development. (A) ABL (Y253F) enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of ABL (Y253F) to determine the potency of the inhibitor (IC₅₀).

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ABL (Y253F) Kinase Enzyme System	Promega	V5086
ADP-Glo™ + ABL (Y253F) Kinase Enzyme System	Promega	V5087

ABL (Y253F) Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 2mM MnCl₂; 50 μ M DTT.