FYN A Kinase Assay

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

Scientific Background:

FYN A (FYN isoform A) is a member of the SRC tyrosine kinase oncogene family showing high homology to YES1, FGR and SRC. FYN has been shown to phosphorylate Dab1, an intracellular adaptor protein that interacts with amyloid precursor protein (APP) and apoE receptor 2 (apoEr2) (1). The interaction of FYN and Dab1 regulates the phosphorylation, trafficking, and processing of APP and apoEr2. FYN expression has been shown to be significantly increased in Chronic Myelogenous Leukemia (CML) (2). Knockdown of FYN with shRNA slows leukemia cell growth, inhibits clonogenicity, and leads to increased sensitivity to imatinib.

1. Hoe, H S. et al: Fyn modulation of Dab1 effects on amyloid precursor protein and ApoE receptor 2 processing. J. Biol. Chem. 2008 Mar 7;283(10):6288-99.

2. Ban, K. et al: BCR-ABL1 mediates up-regulation of Fyn in chronic myelogenous leukemia. Blood, 2008 Mar 1;111(5):2904-8.

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.

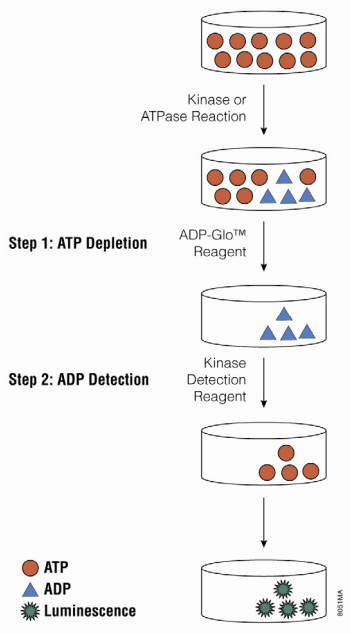


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

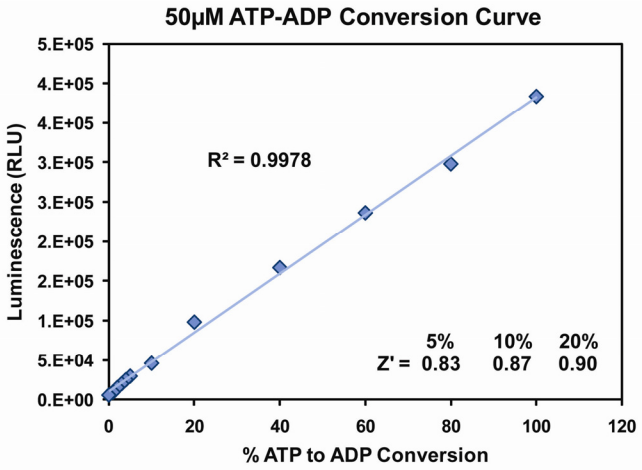


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

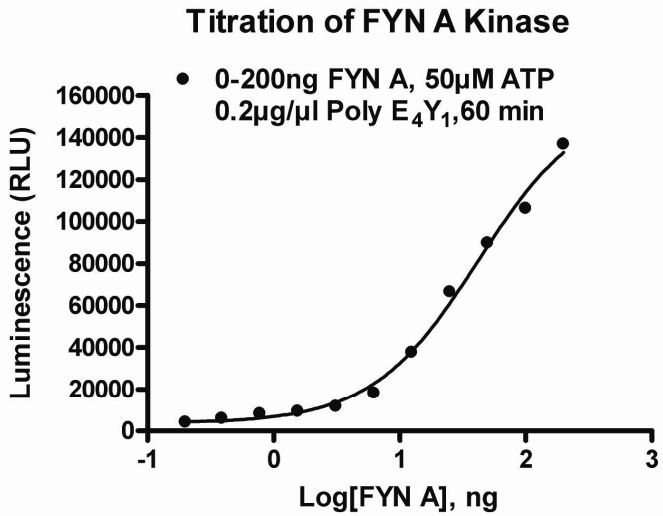
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. FYN 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.

FYN A, ng	100.00	50.00	25.00	12.50	6.25	3.13	1.56	0.78	0.39	0.00
Luminescence	128080	113261	101301	82258	57307	41141	27200	17241	10748	2416
S/B	53.0	46.9	41.9	34.0	23.7	17.0	11.3	7.1	4.4	1.0
% Conversion	92.0	80.9	71.9	57.7	39.0	26.9	16.5	9.0	4.2	0.0



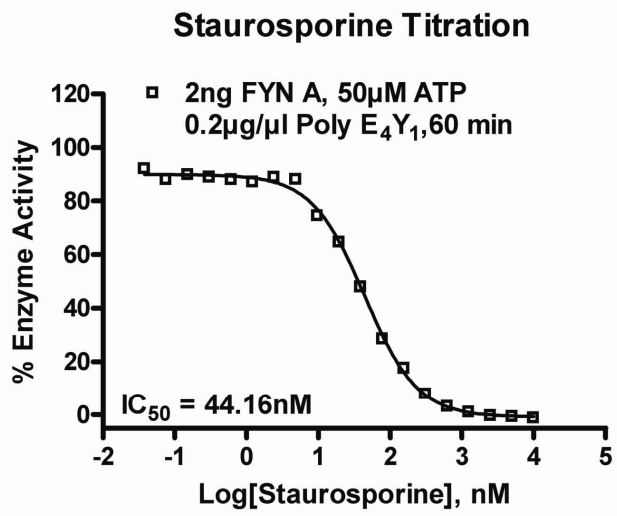


Figure 3. FYN A Kinase Assay Development: (A) FYN A enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of FYN A to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
FYN A Kinase Enzyme System	Promega	V3571
ADP-Glo + FYN A Kinase Enzyme System	Promega	V9341

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