ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: LYN B

LYN B Kinase Assay

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Scientific Background:

LYN is a 56 kd tyrosine kinase that is similar to mouse T-lymphocyte-specific tyrosine kinase p56lck, v-yes, v-fgr and v-src and is expressed in a variety of tissues (1). LYN is expressed preferentially in B cells and can be coimmunoprecipitated with IgM suggesting that LYN is physically associated with membrane-bound IgM, and participates in antigen-mediated signal transduction (2). Crosslinking of membrane-bound IgM with antibody induces rapid increase in activities of LYN and LYN-associated phosphatidylinositol 3-kinase.

1. Yamanashi, Y. et al: The yes-related cellular gene lyn encodes a possible tyrosine kinase similar to p56lck. Mol Cell Biol. 1987 Jan;7(1):237-43.

2. Yamanashi, Y. et al: Association of B cell antigen receptor with protein tyrosine kinase Lyn. Science. 1991 Jan 11;251(4990):192-4.

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.

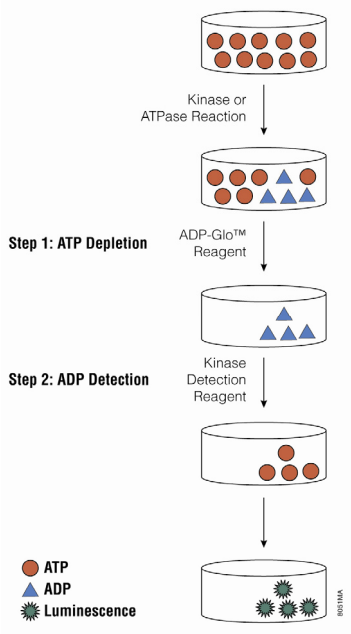

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

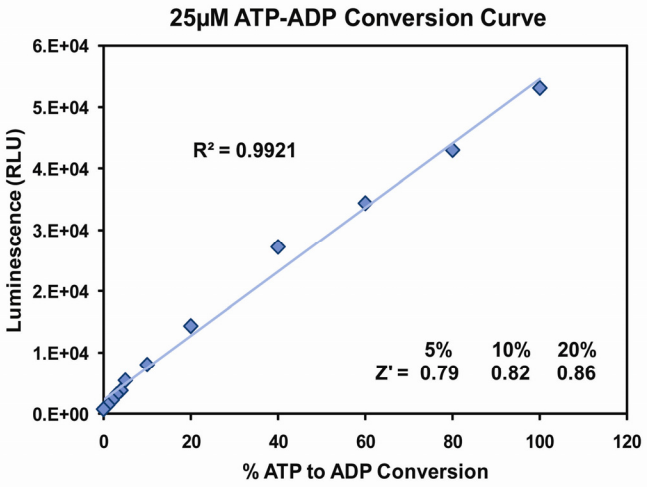

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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. LYN B 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.

LYN B, ng	100	50	25	12.5	6.3	3.13	1.56	0.78	0.39	0
Luminescence	78385	70005	60289	43855	26752	16136	8710	4620	3664	1481
S/B	52.9	47.3	40.7	29.6	18.1	10.9	5.9	3.1	2.5	1.0
% Conversion	65.17	57.92	49.51	35.28	20.47	11.28	4.85	1.31	0.48	0.00

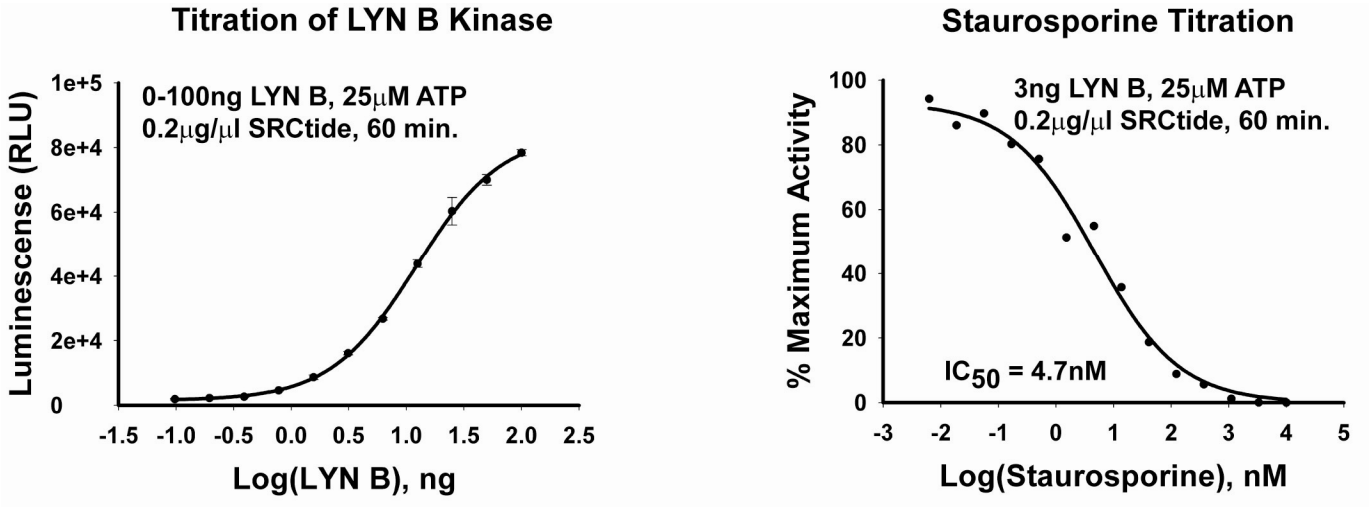

Figure 3. LYN B Kinase Assay Development: (A) LYN B enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 3ng of LYN B to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
LYN B Kinase Enzyme System	Promega	V3711
ADP-Glo + LYN B Kinase Enzyme System	Promega	V9501

LYN B Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT and 2mM $MnCl_2$.