ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

LYN A Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

LYN A is a member of the Src-family of protein kinases that participate in signaling pathways of a variety of cell surface receptors and are localized to the cytoplasmic side of the plasma membrane (1). LYN A is biosynthetically transported to the plasma membrane via the Golgi pool of caveolin along the secretory pathway. The trafficking of LYN A from the Golgi apparatus to the plasma membrane is inhibited by deletion of the kinase domain but not by kinase inactivation. The kinase domain of LYN A plays a role in LYN A trafficking besides catalysis of substrate phosphorylation. cDNA microarray analysis revealed that LYN A may be involved in the changes that are related to tumor pathogenesis (2).

1. Kasahara, K. et al: Trafficking of Lyn through the Golgi caveolin involves the charged residues on alphaE and alphaI helices in the kinase domain. J. Cell Biol., 2004;165(5):641-52.

2. Baran, C.P. et al: The inositol 5'-phosphatase SHIP-1 and the Src kinase Lyn negatively regulate macrophage colony stimulating factor-induced Akt activity. J. Biol. Chem. 2003; 278(40):38628-36

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.

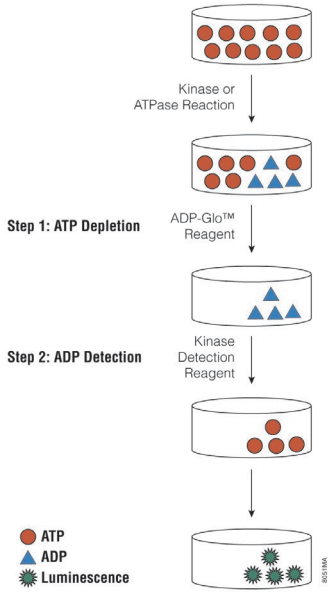

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

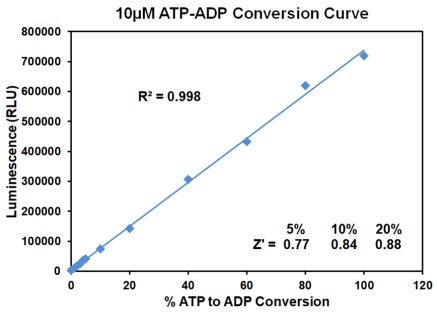

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Tyrosine Kinase Series

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	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0
Luminescence	291,740	244,771	222,289	177,430	129,136	92,342	60,437	36,369	24,089	13,427	8,934	3,700
S/B	79	66	60	48	35	25	16	10	7	4	2	1
% Conversion	67	56	51	41	30	21	14	8	5	3	2	0

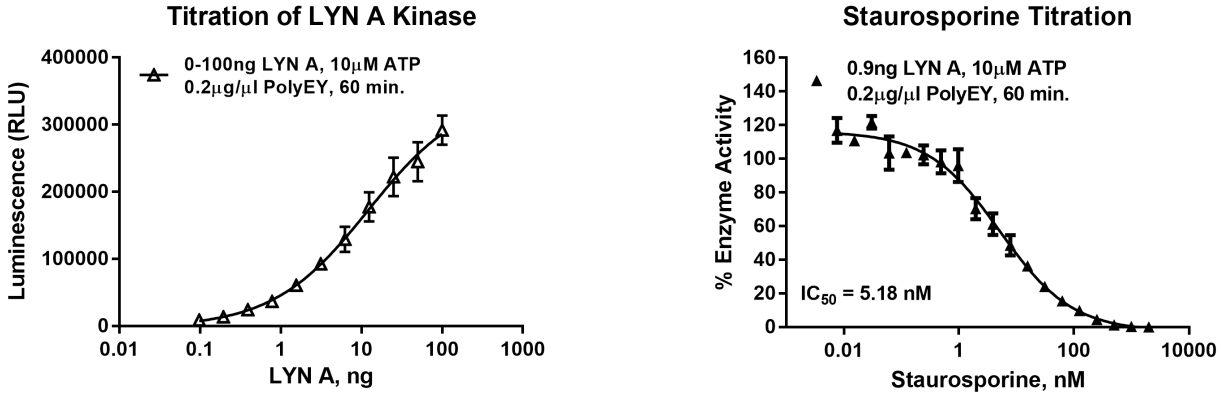

Figure 3. LYN A Kinase Assay Development. (A) LYN A enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 0.9ng of LYN A to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
LYN A Kinase Enzyme System	10µg	VA7486
	1mg	VA7487
ADP-Glo™ + LYN A Kinase Enzyme System	1 Each	VA7488