

ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: SYK



SYK Kinase Assay

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Scientific Background:

SYK is a non-receptor protein tyrosine kinase that is widely expressed in hematopoietic cells. It is involved in coupling activated immunoreceptors to downstream signaling events that mediate diverse cellular responses, including proliferation, differentiation, and phagocytosis. In B cells, SYK plays a crucial role in intracellular signal transduction induced by oxidative stress as well as antigen receptor engagement (1). SYK has been shown to act as a potential tumor suppressor in breast cancer. Absence of SYK protein in primary breast tumors is correlated with poor outcomes. SYK deficient cells have increased motility that is restored to normalcy by replacement with wild-type SYK (2).

1. Takano, T. et al: Role of protein-tyrosine kinase syk in oxidative stress signaling in B cells. *Antioxid Redox Signal*. 2002 Jun;4(3):533-41.
2. Navara, C S.: The spleen tyrosine kinase (Syk) in human disease, implications for design of tyrosine kinase inhibitor based therapy. *Curr Pharm Des*. 2004;10(15):1739-44.

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.

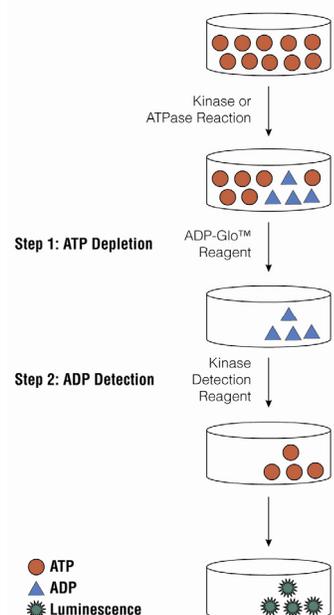


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

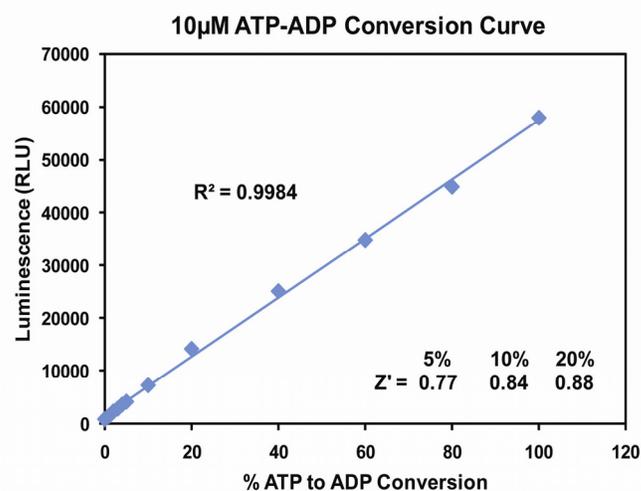


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.



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 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 0.5-1second).

Table 1. SYK 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.

SYK, ng	50	25	12.5	6.25	3.1	1.56	0.78	0.39	0.20	0
Luminescence	66085	63521	51595	25469	14149	5818	2831	1947	1242	1171
S/B	56.4	54.2	44.1	21.7	12.1	5.0	2.4	1.7	1.1	1.0
% Conversion	115.12	110.57	89.41	43.06	22.97	8.19	2.90	1.33	0.08	0.00

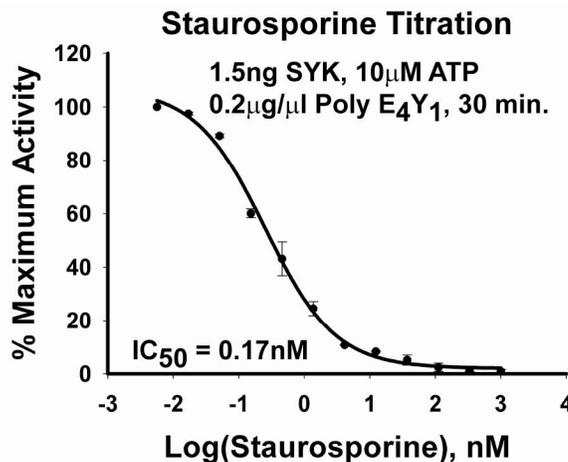
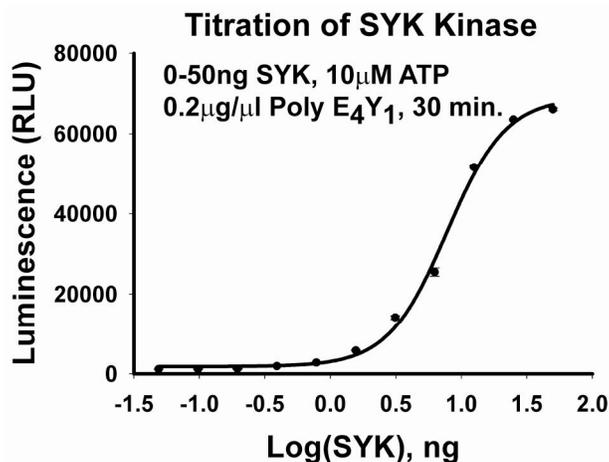


Figure 3. SYK Kinase Assay Development: (A) SYK enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1.5ng of SYK to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
SYK Kinase Enzyme System	Promega	V3801
ADP-Glo + SYK Kinase Enzyme System	Promega	V8271

SYK Kinase Buffer: 40mM Tris, 7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50 μ M DTT.