

ZAP70 Kinase Assay

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Scientific Background:

ZAP70 is a non-receptor protein tyrosine kinase (part of the Syk/Zap70 family) that is involved in signaling by the T-cell antigen receptor (TCR). Ligation of the TCR/CD3 receptor in Jurkat T-cells induces phosphoprotein complexes which contain ZAP70 (1). TCR zeta chains are initially phosphorylated by p56Lck that lead to the recruitment of ZAP70 via its SH2 domain. ZAP70 in turn phosphorylates other proteins in the TCR-phosphoprotein complex. One of the natural substrates for ZAP70 is the zeta-chain dimer of the TCR/CD3 complex (2).

1. Duplay, P. et al: p56lck interacts via its src homology 2 domain with the ZAP-70 kinase. *J Exp Med.* 1994 Apr 1;179(4):1163-72.
2. Chan, A C. et al: Differential expression of ZAP-70 and Syk protein tyrosine kinases, and the role of this family of protein tyrosine kinases in TCR signaling. *J Immunol.* 1994 May 15;152(10):4758-66.

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.

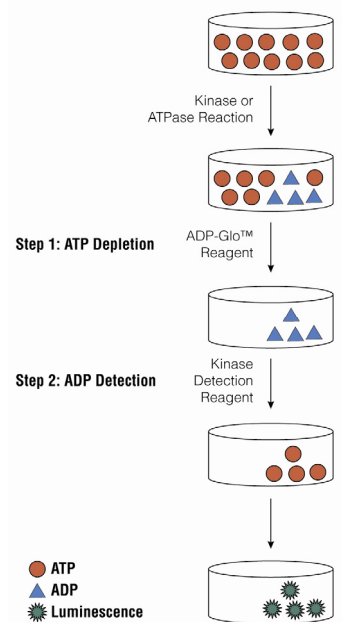


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

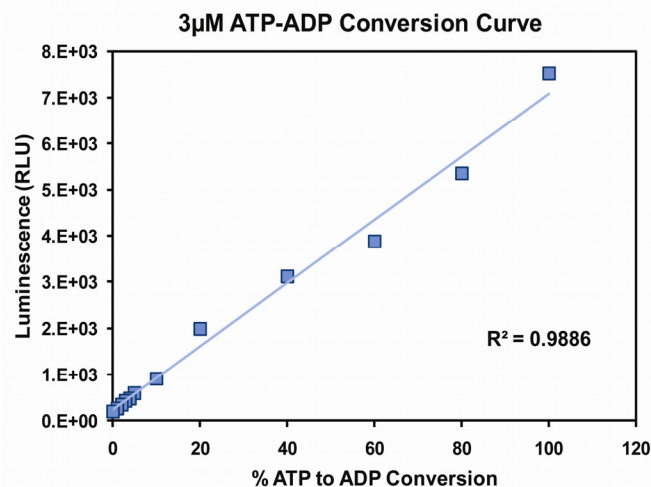


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 3μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction.

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 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 0.5-1second).

Table 1. ZAP70 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.

ZAP70, ng	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.20	0
Luminescence	6855	6539	7790	5508	2411	1337	744	575	383	227
S/B	30.2	28.8	34.3	24.3	10.6	5.9	3.3	2.5	1.7	1
% Conversion	96.7	92.1	110.3	77.0	31.8	16.1	7.5	5.0	2.2	0

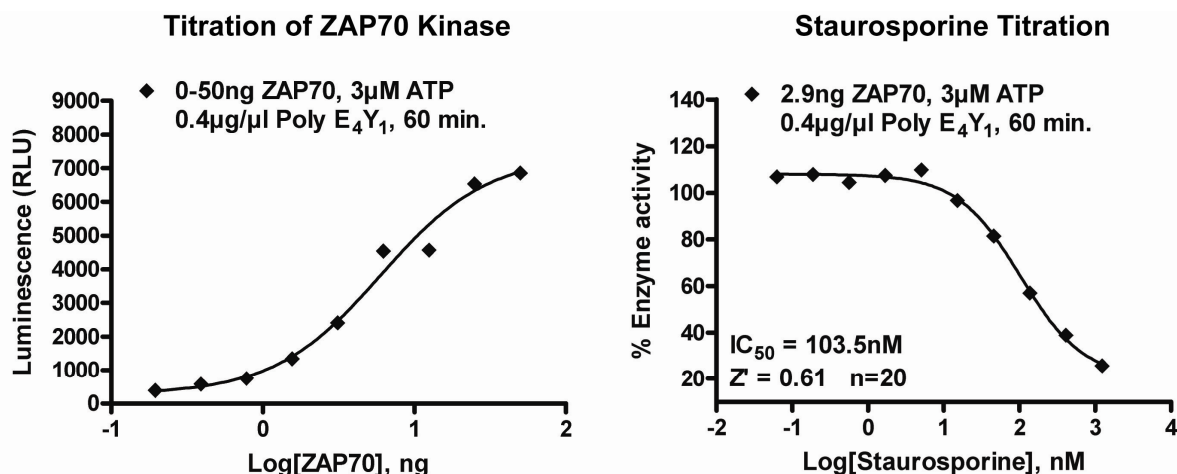


Figure 3. ZAP70 Kinase Assay Development. (A) ZAP70 enzyme was titrated using 30 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2.9ng of ZAP70 to determine the potency of the inhibitor (IC₅₀). Z' factor was determined using 20 replicates of each of the minimum and maximum response (10 and 0 μ M staurosporine, respectively).

Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ZAP70 Kinase Enzyme System	Promega	V3811
ADP-Glo + ZAP70 Kinase Enzyme System	Promega	V8311

ZAP70 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 2mM MnCl₂; 50 μ M DTT.