ITK Kinase Assay

By Jolanta Vidugiriene, Ph.D., Hicham Zegzouti, Ph.D., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

ITK is a member of the TEC family of non-receptor tyrosine kinases. ITK is expressed in T-cells and is important for T-cell development and activation through the antigen receptor. ITK requires prior activation of Lck, Zap-70 and PI3-kinase for efficient activation and shares major substrates with both Lck and Zap-70 (1). ITK knockout mice show multiple effects on T cell development, cytokine production and T-helper cell differentiation. T cells that lack or express mutant versions of ITK show impaired TCR-induced actin polymerization, cell polarization and regulation of the signaling events involved in cytoskeletal reorganization (2).

1. August, A. et al: The Tec family of tyrosine kinases in T cells, amplifiers of T cell receptor signals. Int J Biochem Cell Biol. 2002 Oct;34(10):1184-9.

2. Finkelstein, L D. et al: Tec kinases: shaping T-cell activation through actin. Trends Cell Biol. 2004 Aug;14(8):443-51.

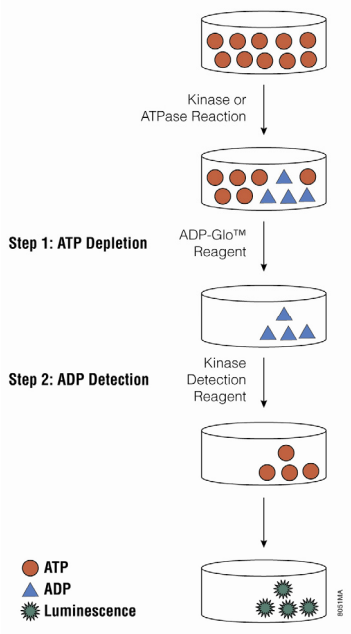


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

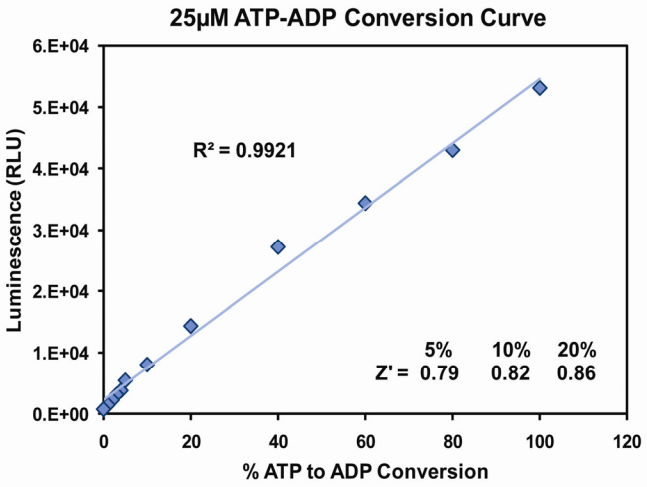


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

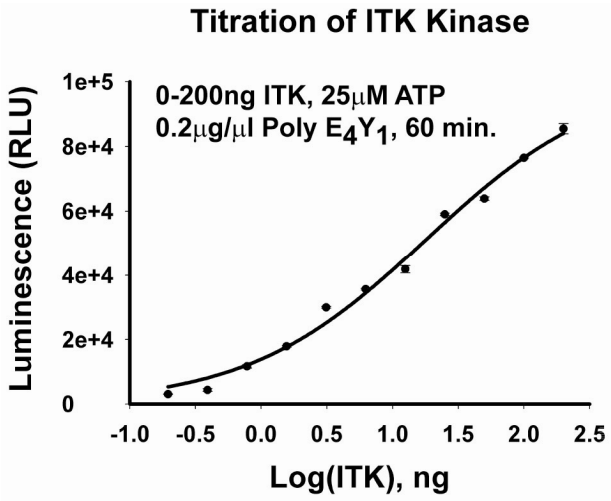
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. ITK 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.

ITK, ng	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
Luminescence	76494	63899	59053	41875	35632	29993	17909	11683	4371	1561
S/B	49.0	40.9	37.8	26.8	22.8	19.2	11.5	7.5	2.8	1
% Conversion	63.5	52.6	48.4	33.6	28.2	23.3	12.8	7.4	1.1	0



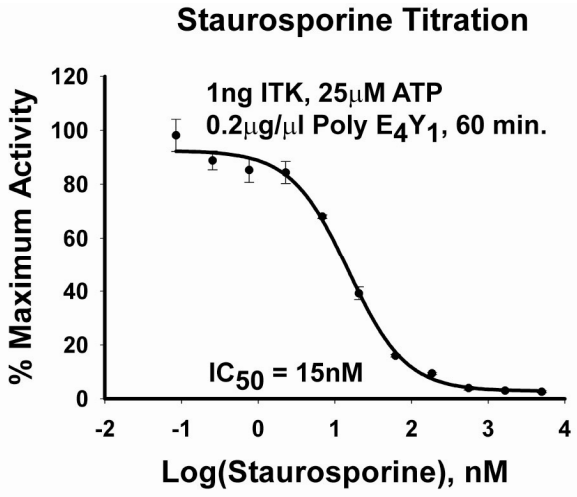


Figure 3. ITK Kinase Assay Development: (A) ITK enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of ITK to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ITK Kinase Enzyme System	Promega	V3191
ADP-Glo + ITK Kinase Enzyme System	Promega	V9431

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