ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: IRAK4

IRAK4 Kinase Assay

By Hicham Zegzouti, Ph.D., Jolanta Vidugiriene, Ph.D., Kevin Hsiao, M.S., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

Interleukin-1 receptor-associated kinase 4 (IRAK4) is an important mediator in the signal transduction of Toll-like receptor (TLR) and IL1R family members (1). IRAK4 is involved in the Toll-like receptor signaling pathway leading to Apoptosis. IRAK4 has molecular functions like protein binding, ATP binding, kinase activity and magnesium ion binding. Toll/IL-1 receptor family members like IRAK4 are central components of host defense mechanisms in a variety of species (2). One well conserved element in their signal transduction is the Ser/Thr kinase activity which couple early signaling events in a receptor complex at the plasma membrane to larger signalosomes in the cytosol.

1. Li, S. et al: IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. Proc. Natl. Acad. Sci. USA. 2002; 99:5567-72.

2. Suzuki, N. et al: IRAK-4 as the central TIR signaling mediator in innate immunity. Trends Immunol. 2002; 23:503-6

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.

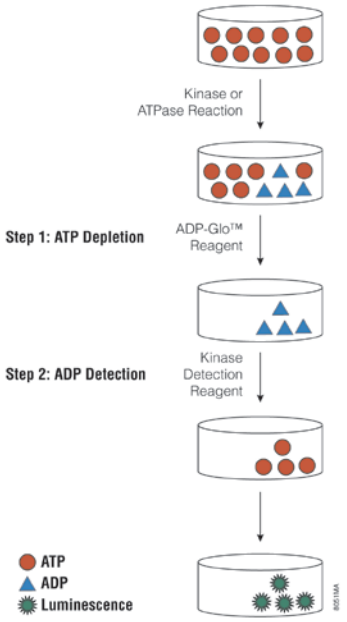

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

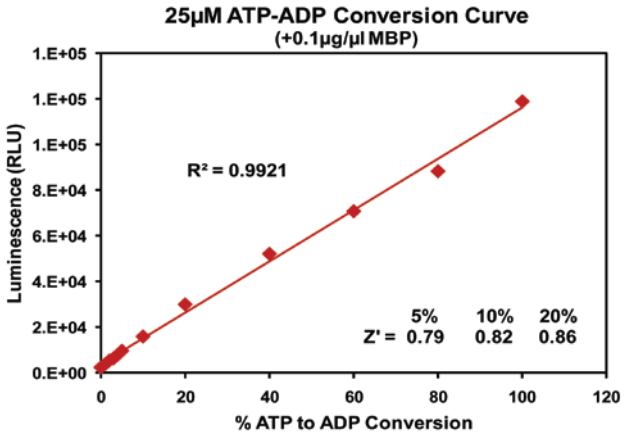

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. IRAK4 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.

IRAK4, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	80024	67888	49208	34071	23025	15273	9842	6673	5213	2231
S/B	35.9	30.4	22.1	15.3	10.3	6.8	4.4	3.0	2.3	1
% Conversion	69.2	58.1	41.1	27.3	17.2	10.1	5.2	2.3	1.8	0

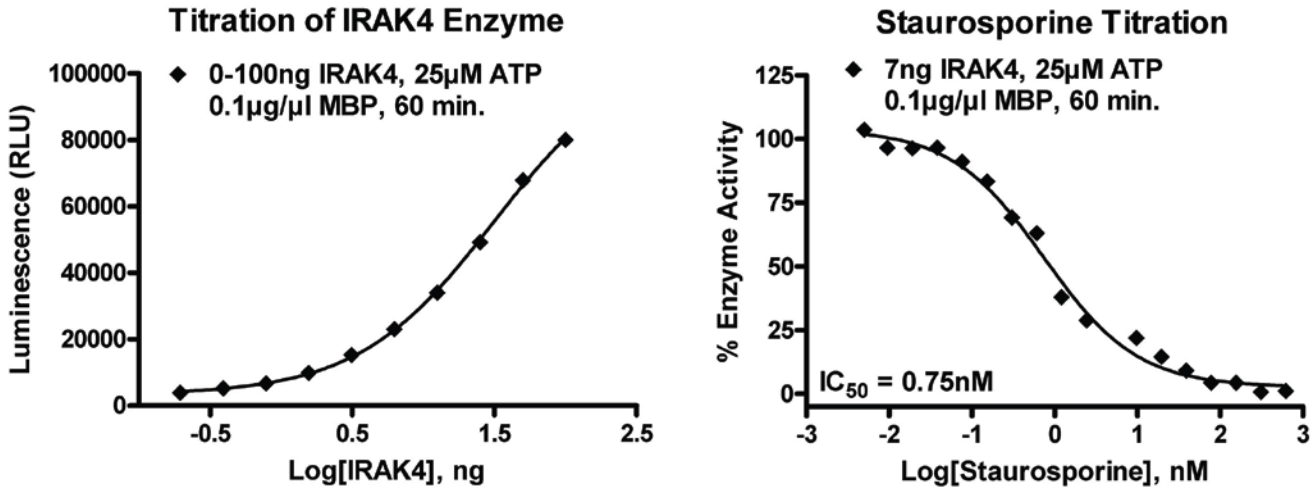

Figure 3. IRAK4 Kinase Assay Development. (A) IRAK4 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 7ng of IRAK4 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
IRAK4 Kinase Enzyme System	Promega	V2621
ADP-Glo™ + IRAK4 Kinase Enzyme System	Promega	V9421

IRAK4 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.