ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

IRAK1 Kinase Assay

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

Scientific Background:

IRAK1 encodes the interleukin-1 receptor-associated kinase 1, one of two putative serine/threonine kinases that become associated with the interleukin-1 receptor (IL1R) upon stimulation. The exposure of HeLa cells or human embryonic kidney cells overexpressing IL1R to IL1 caused rapid association of IRAK with the IL1R complex and phosphorylation of IRAK (1). IRAK1 is partially responsible for IL1-induced upregulation of the transcription factor NF-kappa B. IRAK1 as a risk gene with critical role in the pathogenesis of systemic lupus erythematosus (2).

1. Cao, Z. et.al: IRAK: a kinase associated with the interleukin-1 receptor. Science 271: 1128-1131, 1996.

2. Jacob, C. O. et.al: Identification of IRAK1 as a risk gene with critical role in the pathogenesis of systemic lupus erythematosus. Proc. Nat. Acad. Sci. 106: 6256-6261, 2009.

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.

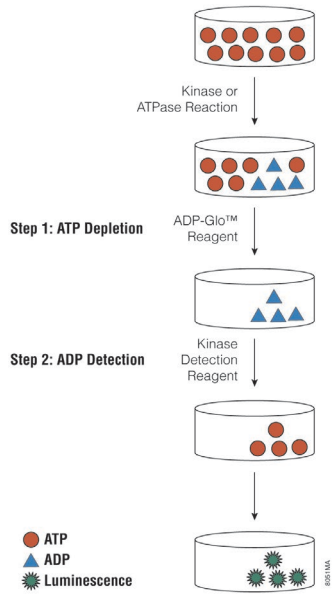

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

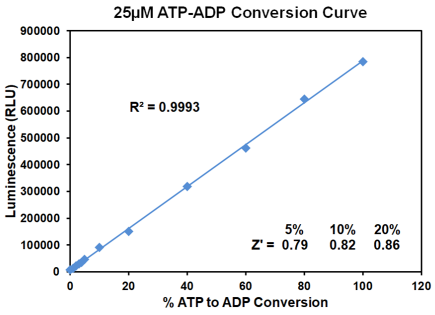

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Ser/Thr 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	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.29	0
Luminescence	665,223	501,842	374,922	200,978	102,239	49,593	25,984	12,836	4,100	2,104
S/B	316	238	178	96	49	24	12	6	2	1
% Conversion	71	53	39	20	10	4	2	0	0	0

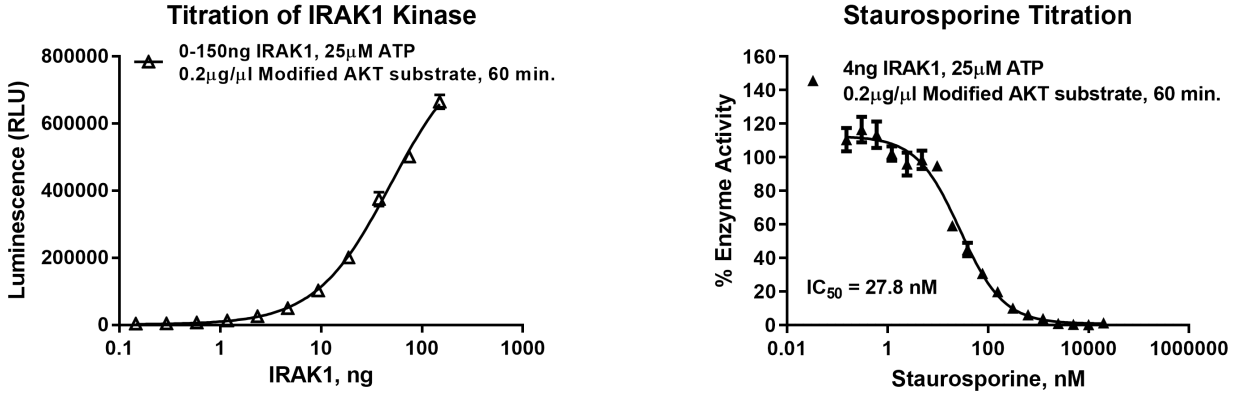

Figure 3. IRAK1 Kinase Assay Development. (A) IRAK1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 4ng of IRAK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
IRAK1 Kinase Enzyme System	10µg	VA7477
	1mg	VA7478
ADP-Glo™ + IRAK1 Kinase Enzyme System	1 Each	VA7479