ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

RIPK1 Kinase Assay

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Scientific Background:

RIPK1 or Receptor Interacting Protein Kinase 1 is a serine/threonine kinase that was originally identified as interacting with the cytoplasmic domain of FAS. RIPK1 has been deemed as an important element in the signal transduction machinery that mediates programmed cell death. RIPK1 has been shown to interact with TRADD, TRAF1 TRAF2 and TRAF3 and TRADD can act as an adaptor protein to recruit RIPK1 to the TNFR1 complex in a TNF-dependent process (1). TNFα is capable of activating the noncanonical NF-κB pathway, but this activation of this pathway is negatively regulated by RIPK1 (2).

1. Hsu, H. et al: TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity 4: 387-396, 1996.

2. Kim, J Y. et al: TNFα induced noncanonical NF-κB activation is attenuated by RIP1 through stabilization of TRAF2. J Cell Sci. 2011 Feb 15;124 (Pt 4):647-56.

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.

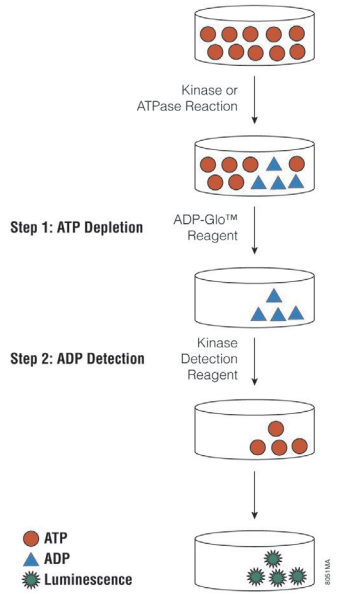

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

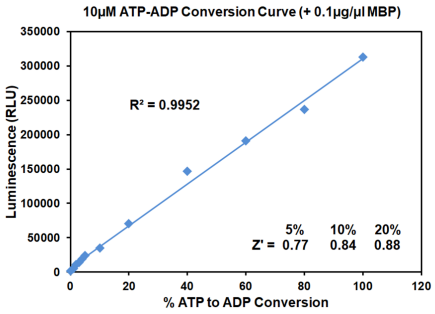

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	2.34	0
Luminescence	90,691	48,649	29,408	12,656	7,228	2,165	1,123
S/B	81	43	26	11	6	2	1
% Conversion	42	22	12	4	2	0	0

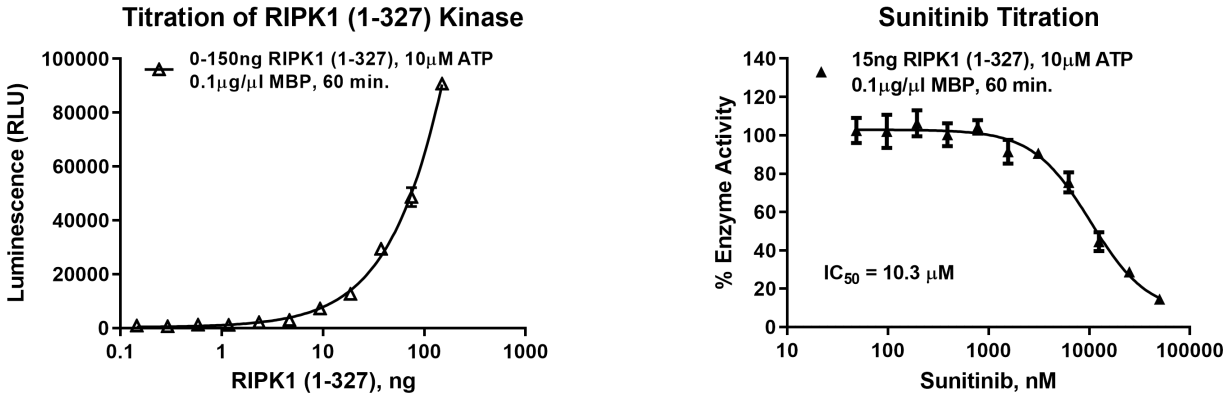

Figure 3. RIPK1 (1-327) Kinase Assay Development. (A) RIPK1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 15ng of RIPK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
RIPK1 Kinase Enzyme System	10µg	VA7591
	1mg	VA7592
ADP-Glo™ + RIPK1 Kinase Enzyme System	1 Each	VA7593