Promega

RIPK2 Kinase Assay

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Scientific Background:

RIPK2 (RIP2; RICK) is a death domain-containing protein kinase encoding a predicted 540-amino acid protein which contains an N-terminal serine/threonine kinase catalytic domain and a C-terminal caspase activation and recruitment domain. RIPK2 is thought to regulate apoptosis induced by the FAS receptor pathway (1). RIPK2 has been shown to specifically interact with the CARD of ICE/caspase-1 and this interaction correlates with the processing of pro-caspase-1 and the formation of the active caspase-1 p20 (2).

1. Inohara, N. et al: RICK, a novel protein kinase containing a caspase recruitment domain, interacts with CLARP and regulates CD95-mediated apoptosis. J. Biol. Chem. 273: 12296-12300, 1998. Note: Erratum: J. Biol. Chem 273: 18675 only, 1998.
2. Thome, M. et al: Identification of CARDIAK, a RIP-like kinase that associates with caspase-1. Curr. Biol. 8: 885-888, 1998.

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.

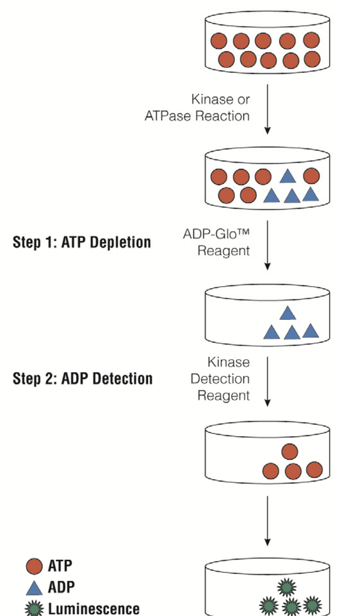


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

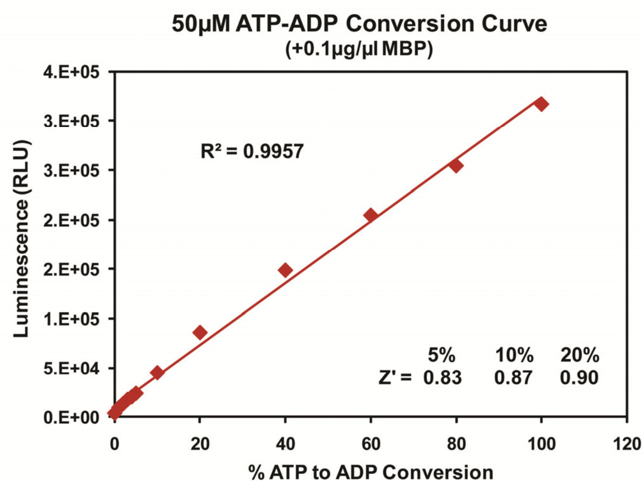
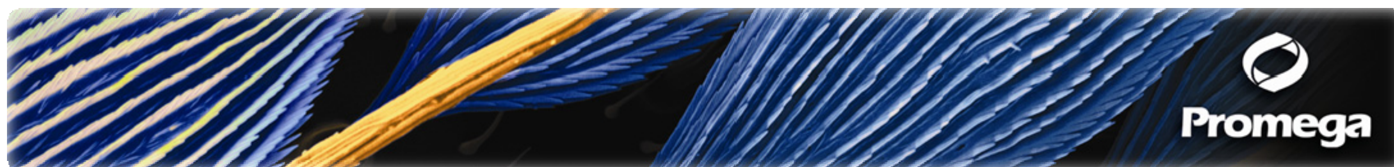


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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. RIPK2 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.

RIPK2, ng	200	100	50	25	12	6.3	3.1	1.6	0.8	0
RLU	170705	93670	57115	31430	16638	8747	4740	2649	1718	909
S/B	187.8	103.0	62.8	34.6	18.3	9.6	5.2	2.9	1.9	1
% Conversion	59.8	32.6	19.7	10.7	5.5	2.7	1.3	0.5	0.2	0

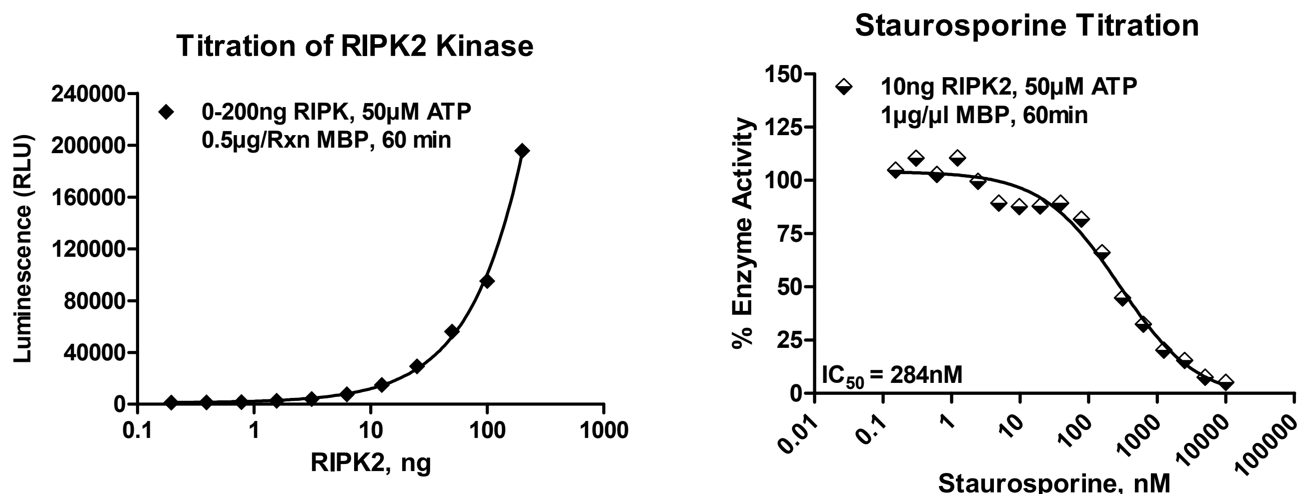


Figure 3. RIPK2 Kinase Assay Development. (A) RIPK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of RIPK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Promega SignalChem Specialists in Signaling Proteins

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
RIPK2 Kinase Enzyme System	Promega	V4084
ADP-Glo™ + RIPK2 Kinase Enzyme System	Promega	V4085

RIPK2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.