ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: JNK1

JNK1 Kinase Assay

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Scientific Background:

JNK1 is a member of the MAP kinase group that is activated by dual phosphorylation at thr and tyr residues during exposure to stress such as UV irradiation. JNK1 binds to the c-Jun transactivation domain and phosphorylates it on Ser-63 and Ser-73 (1). JNK1 has been shown to play an important role in disease processes. Activation of JNK1 results in defects in myotube viability and integrity leading to dystrophic myofiber destruction (2). JNK1 activity is also abnormally elevated in obesity and removal of JNK1 results in decreased adiposity and significantly improved insulin sensitivity.

1. Derijard, B. et al: JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell. 1994 Mar 25;76(6):1025-37.

2. Kolodziejczyk, S M. et al: Activation of JNK1 contributes to dystrophic muscle pathogenesis. Curr Biol. 2001 Aug 21;11(16):1278-82.

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.

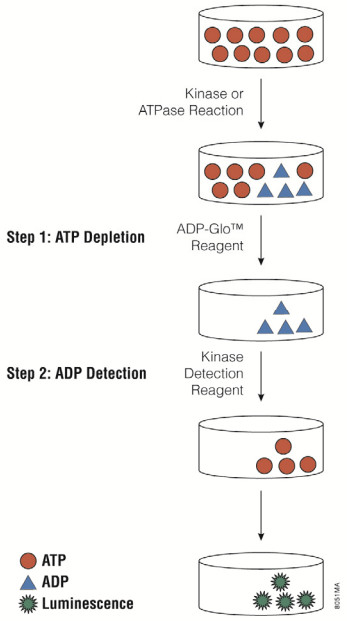

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

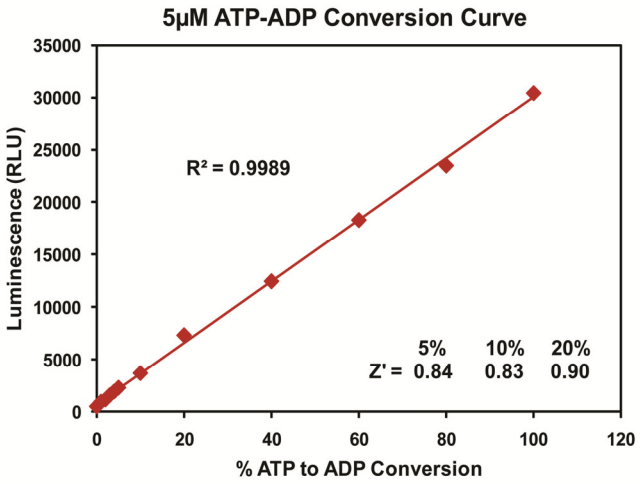

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. JNK1 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.

JNK1, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0
RLU	20227	17036	10220	5644	1794	1029	740	706	340
S/B	59	50	30	17	5.3	3.0	2.18	2.08	1
% Conversion	46	39	23	12	3.4	1.6	0.96	0.88	0

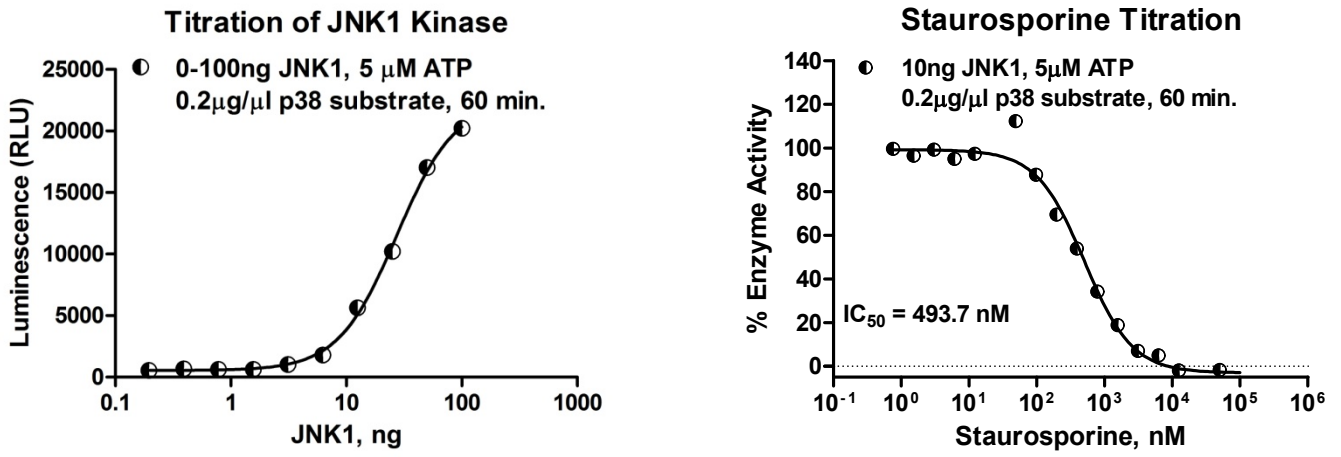

Figure 3. JNK1 Kinase Assay Development. (A) JNK1 enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of JNK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
JNK1 Kinase Enzyme System	Promega	V4070
ADP-Glo™ + JNK1 Kinase Enzyme System	Promega	V4071

JNK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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