JNK3 Kinase Assay

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

Scientific Background:

JNK3 is a member of the c-Jun N-terminal kinases (JNKs) which are part of the mitogen-activated protein (MAP) kinase family, and regulate signal transduction in response to environmental stress. JNK3 phosphorylates various transcription factors such as ATF2, Elk-1 and members of the Jun family (1). Activation and nuclear localization of JNK3, a neuronal-specific isoform of JNK, has been associated with hypoxic and ischemic damage of CA1 neurons in the hippocampus. Knockout mice lacking JNK3 showed reduced apoptosis of hippocampal neurons and reduced seizure induced by kainic acid, a glutamate-receptor agonist (2).

1. Gupta, S. et al: Selective interaction of JNK protein kinase isoforms with transcription factors. EMBO J. 1996 Jun 3; 15(11):2760-70.
2. Yang, D D. et al: Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. Nature. 1997 Oct 23; 389(6653):865-70.

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.

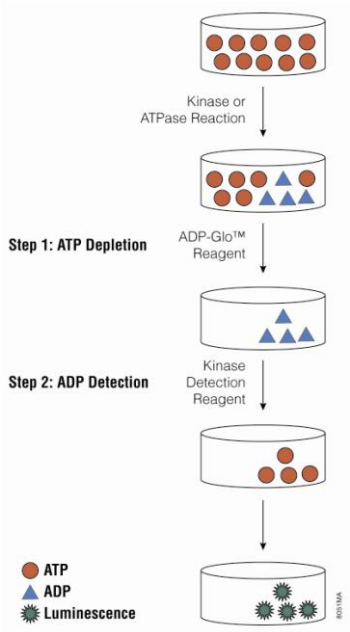

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

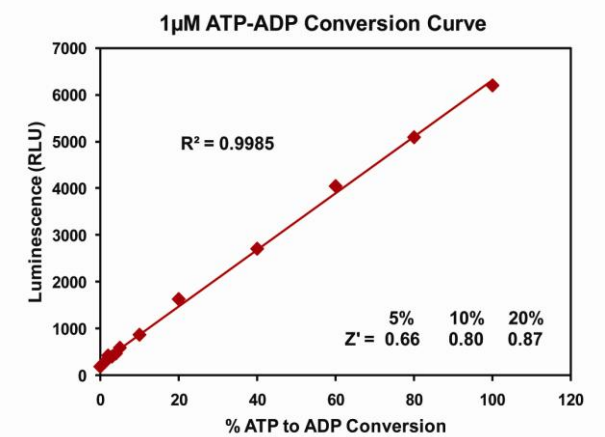

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 1µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. JNK3 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.

JNK3, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	8310	8436	7832	7105	4822	2816	1620	751	587	309
S/B	27	27	25	23	16	9	5	2.4	1.9	1
% Conversion	92	93	86	78	51	28	14	4.4	3.5	0

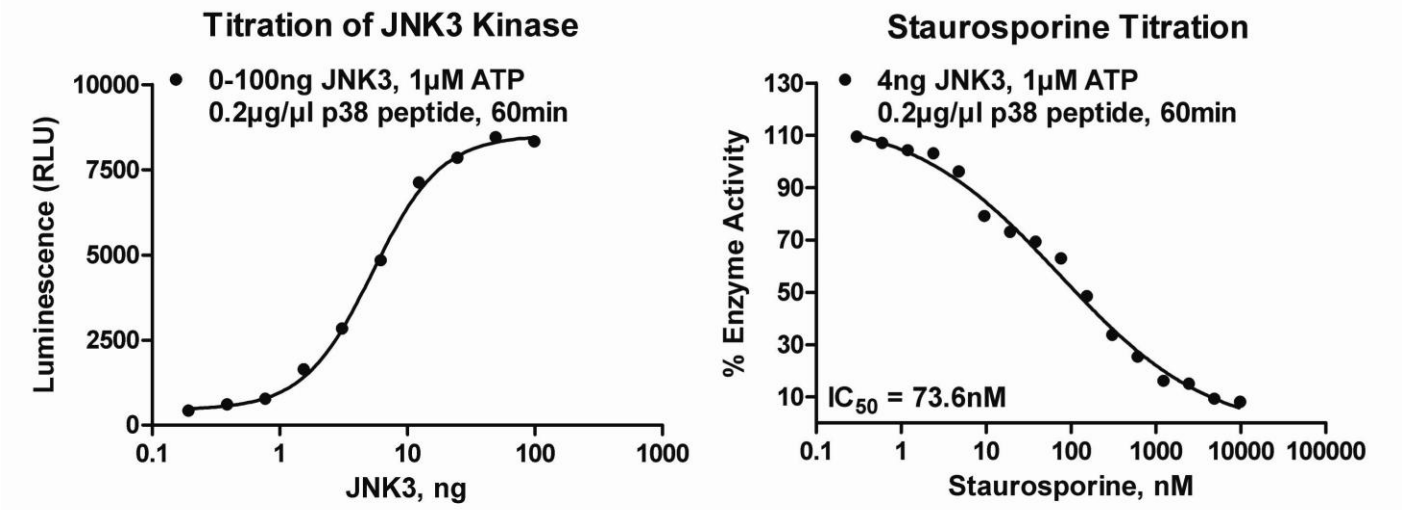

Figure 3. JNK3 Kinase Assay Development. (A) JNK3 enzyme was titrated using 1µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 4ng of JNK3 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
JNK3 Kinase Enzyme System	Promega	V3821
ADP-Glo™ + JNK3 Kinase Enzyme System	Promega	V9461

JNK3 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.