ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

JNK2 Kinase Assay

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Scientific Background:

JNK2 is a member of the JNK family that acts as an integration point for multiple biochemical signals and is involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. JNK2 targets specific transcription factors, and mediates immediate-early gene expression in response to various cell stimuli. Both UV radiation and the proinflammatory cytokine TNFα can induce JNK2. JNK2 play a main role in the regulation of regional specific apoptosis (1) during early brain development and providing protection against autoimmune diabetes (2).

1. Kuan, C. et.al: The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. Neuron 22: 667-676, 1999.

2. Jaeschke, A. et.al: Disruption of the Jnk2 (Mapk9) gene reduces destructive insulitis and diabetes in a mouse model of type I diabetes. Proc. Nat. Acad. Sci. 102: 6931-6935, 2005.

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.

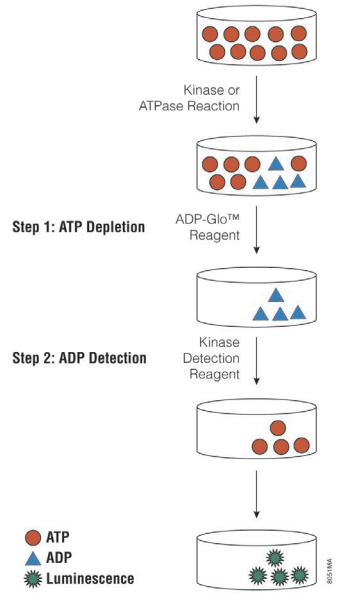

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

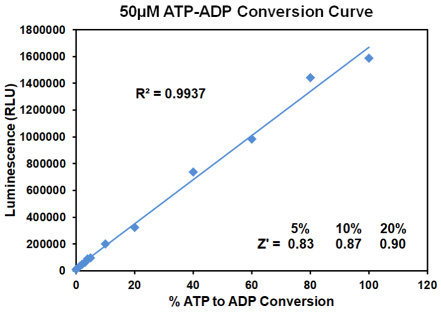

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0
Luminescence	1,515,435	766,135	431,184	290,777	174,567	86,715	48,702	27,850	19,430	8,782
S/B	173	87	49	33	20	10	6	3	2	1
% Conversion	111	56	31	20	12	5	2	1	0	0

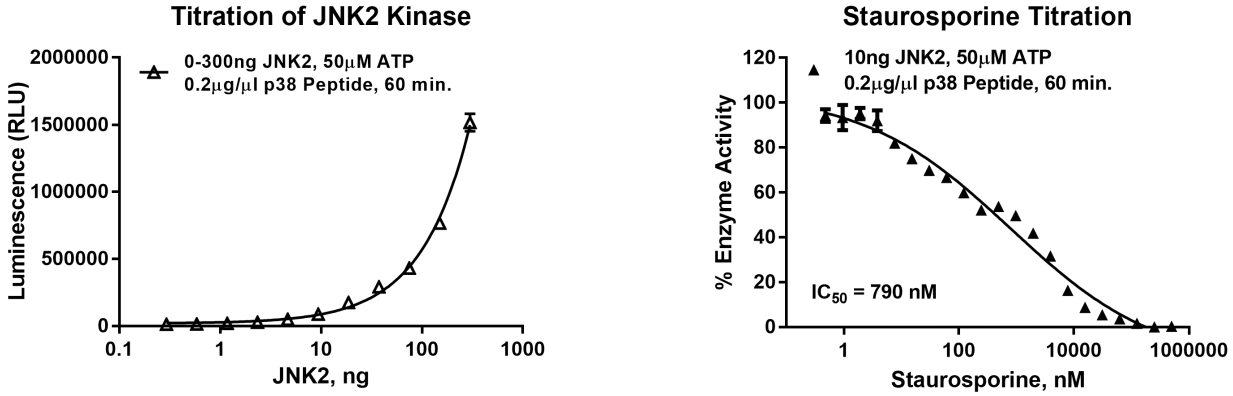

Figure 3. JNK2 Kinase Assay Development. (A) JNK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 10ng of JNK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
JNK2 Kinase Enzyme System	10µg	VA7210
	1mg	VA7211
ADP-Glo™ + JNK2 Kinase Enzyme System	1 Each	VA7212