ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

JAK1 Kinase Assay

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Scientific Background:

JAK1 is a member of protein-tyrosine kinases (PTK) characterized by the presence of a second phosphotransferase-related domain immediately N-terminal to the PTK domain. JAK1 bears all the hallmarks of a protein kinase, although its structure differs significantly from that of the PTK and threonine/serine kinase family members. JAK1 is a large, widely expressed membrane-associated phosphoprotein that is involved in the interferon-alpha/beta and -gamma signal transduction pathways (1). JAK1 plays an essential and nonredundant role in promoting biologic responses induced by a select subset of cytokine receptors, including those in which JAK utilization is thought to be nonspecific (2).

1. Muller, M.et.al: The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. Nature 366: 129-135, 1993.

2. Rodig, S. J.et.al: Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell 93: 373-383, 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.

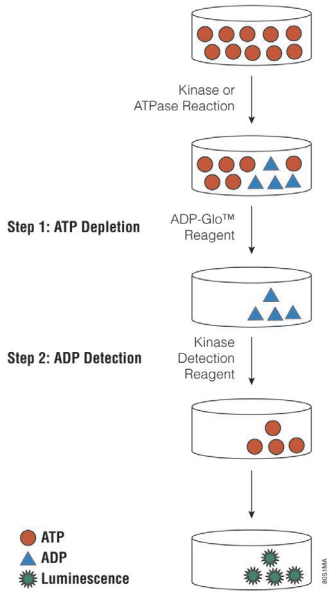

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

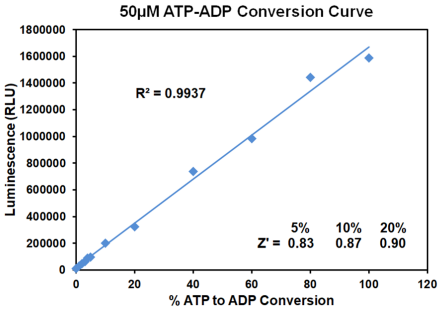

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	0
Luminescence	1,072,933	647,286	340,863	137,845	60,130	27,109	14,069	6,326
S/B	170	102	54	22	10	4	2	1
% Conversion	82	49	26	10	4	2	1	0

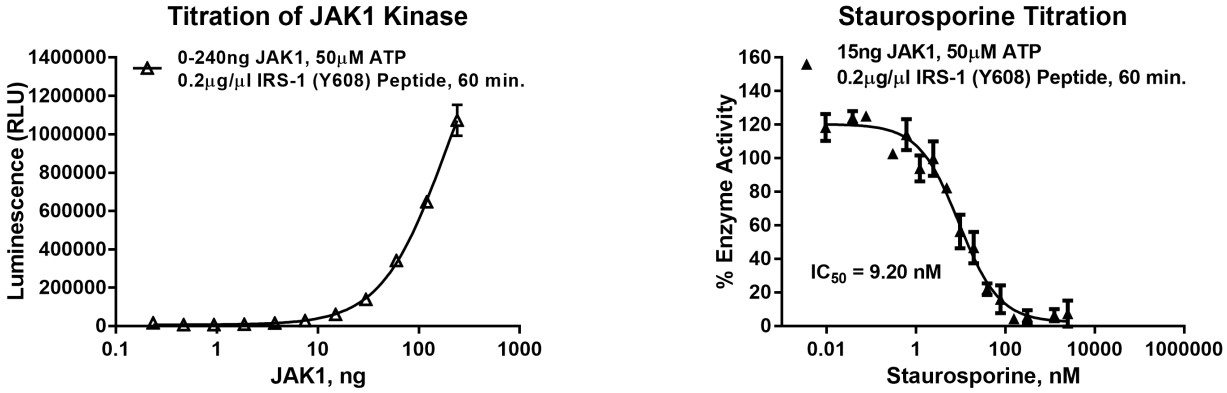

Figure 3. JAK1 Kinase Assay Development. (A) JAK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 15ng of JAK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
JAK1 Kinase Enzyme System	10µg	VA7207
	1mg	VA7208
ADP-Glo™ + JAK1 Kinase Enzyme System	1 Each	VA7209