JAK3 Kinase Assay

By Jolanta Vidugiriene, Ph.D., Hicham Zegzouti, Ph.D., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

JAK3 is a member of the JAK family of tyrosine kinases involved in cytokine receptor-mediated intracellular signal transduction. Low levels of JAK3 expression is detected in immature hematopoietic cells, which dramatically increases during terminal differentiation of these cells suggesting a role of JAK3 in the differentiation of hematopoietic cells. Mutations in JAK3 are associated with autosomal SCID (severe combined immunodeficiency disease) (1). Mice lacking JAK3 show a severe block in B-cell development at the pre-B stage in bone marrow suggesting that JAK3 is critical for the progression of B-cell development in the bone marrow (2).

1. Russell S M, et al: Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science 270: 797-799, 1995.

2. Thomis D C, et al: Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science 270: 794-797, 1995.

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.

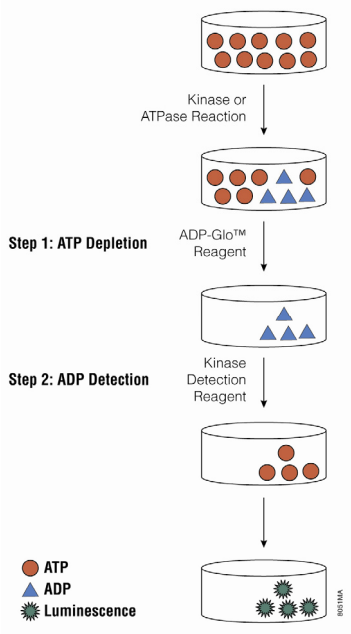

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

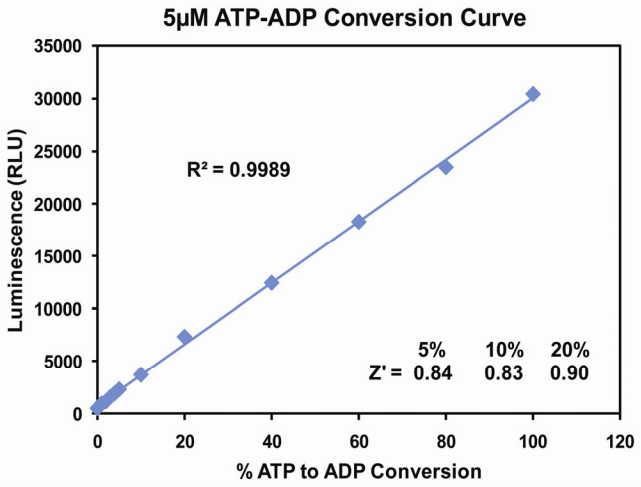

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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. JAK3 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.

JAK3, ng	25	12.5	6.25	3.1	1.56	0.78	0.39	0.20	0.10	0
Luminescence	44522	38620	35477	15186	6364	3230	1942	1713	881	563
S/B	79.1	68.7	63.1	27.0	11.3	5.7	3.5	3.0	1.6	1.0
% Conversion	149.54	129.36	118.62	49.27	19.11	8.40	4.00	3.22	0.37	0.00

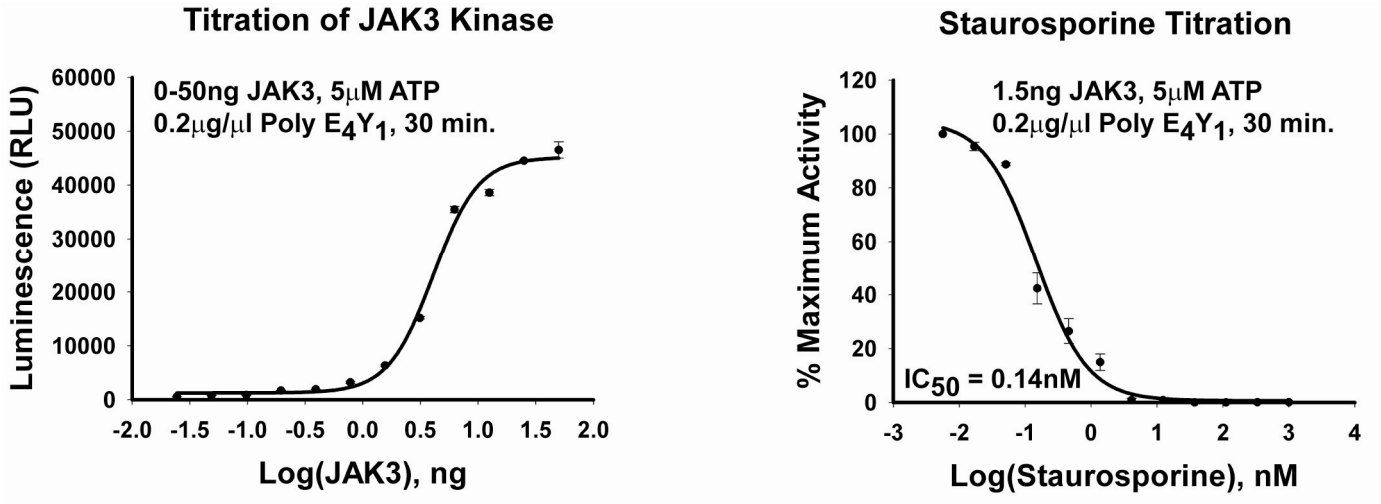

Figure 3. JAK3 Kinase Assay Development: (A) JAK3 enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1.5ng of JAK3 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
JAK3 Kinase Enzyme System	Promega	V3701
ADP-Glo + JAK3 Kinase Enzyme System	Promega	V9441

JAK3 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.