KHS1 Kinase Assay

By Juliano Alves, Ph.D., Mary Sobol, M.S., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

KHS1 belongs to serine/threonine kinase family that has a STE20-like protein kinase domain which stimulates the stress-activated protein kinase (SAPK, also known as Jun kinase or JNK) pathway (1). It is a mitogen-activated protein kinase kinase kinase kinase 5, termed germinal center kinase related (GCKR). Recently, the KH domain of Escherichia coli polynucleotide phosphorylase has been reported to be necessary for autoregulation and growth at low temperature (2).

1. Shi, C.S. et al: TNF-mediated activation of the stress-activated protein kinase pathway: TNF receptor-associated factor 2 recruits and activates germinal center kinase related. J. Immunol. 1999;163(6):3279-85.

2. Maura Epifanía, M.O. et al: The KH and S1 domains of Escherichia coli polynucleotide phosphorylase are necessary for autoregulation and growth at low temperature. Biochim. et Biophy. Acta (BBA). 2007; 1769 (3):194-203

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.

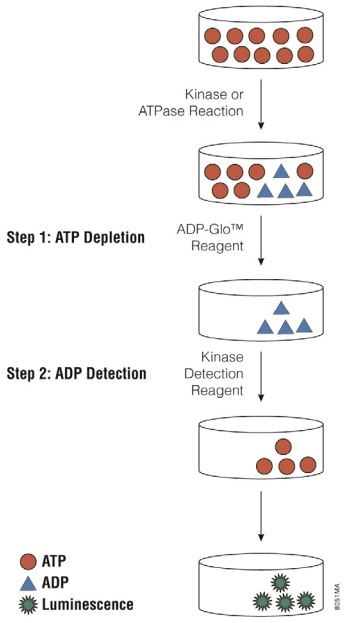

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

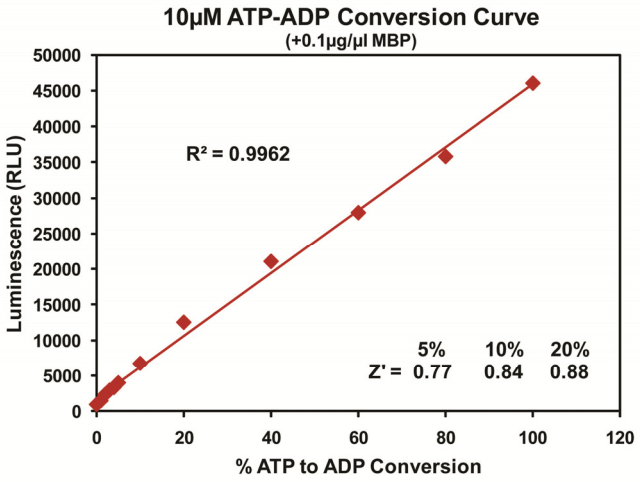

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 120 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. KHS1 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.

KHS1, ng	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0
RLU	66377	64524	51762	38062	18957	9959	4889	2779	1385	518
S/B	128	124	100	73	37	19	9	5	3	1
% Conversion	96	93	74	54	26	13	6	2	0.4	0

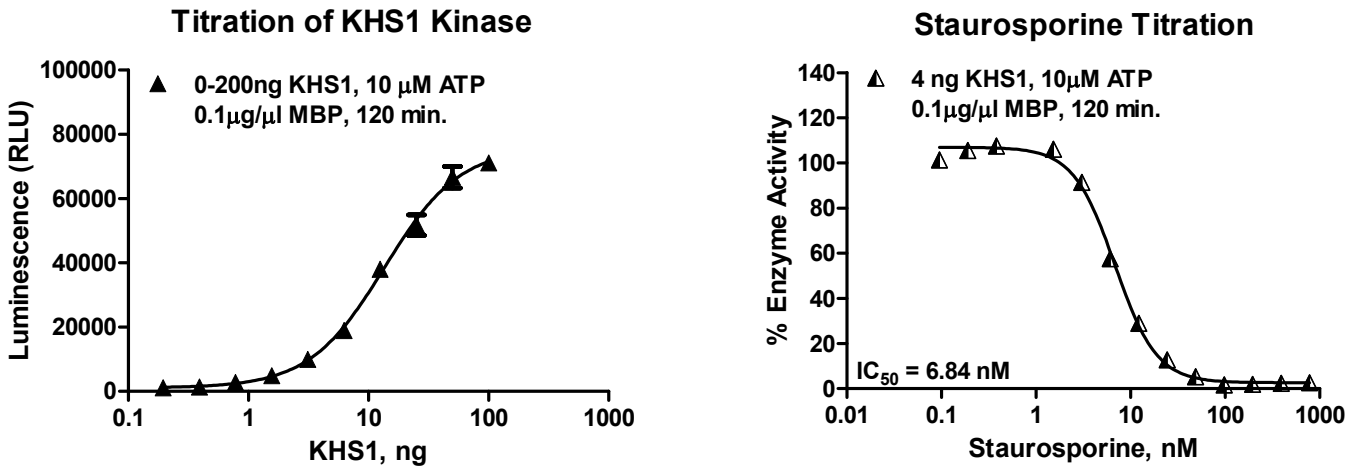

Figure 3. KHS1 Kinase Assay Development. (A) KHS1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 4ng of KHS1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
KHS1 Kinase Enzyme System	Promega	V4108
ADP-Glo™ + KHS1 Kinase Enzyme System	Promega	V4109

KHS1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
promega.com