ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: MSK2

MSK2 Kinase Assay

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

Scientific Background:

MSK2 or mitogen- and stress-activated protein kinase-2 is a member of the serine/threonine kinases family that contains 2 non-identical kinase catalytic domains and phosphorylates various substrates, including CREB1 and c-fos (1). MSK2 is also known as ribosomal protein S6 kinase 4 (RPS6KA4) that are activated by the mitogen-activated protein kinases ERK1, ERK2, and p38 (2).

1. Pierrat, B. et.al: RSK-B, a novel ribosomal S6 kinase family member, is a CREB kinase under dominant control of p38-alpha mitogen-activated protein kinase (p38-alpha-MAPK). J. Biol. Chem. 273: 29661-29671, 1998.

2. Deak, M. et.al: Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. EMBO J. 17: 4426-4441, 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.

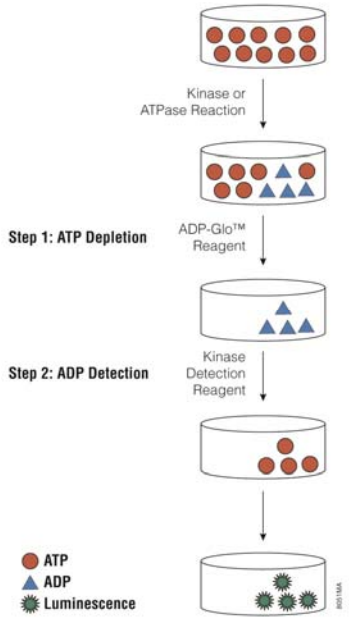

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

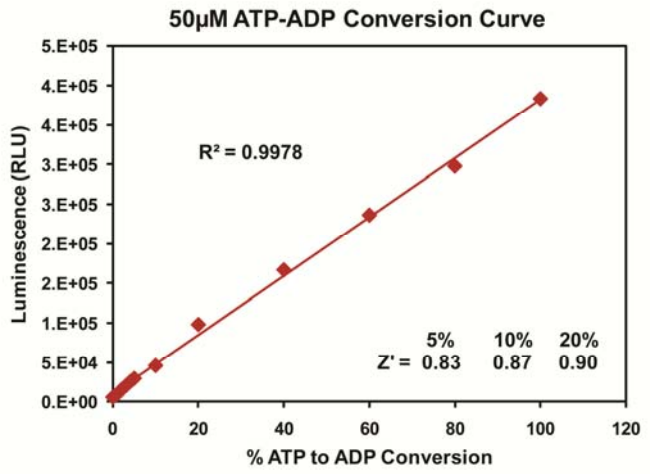

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

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 0.5-1sec).

Table 1. MSK2 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.

MSK2, ng	200	100	50	25	12.5	6.3	3.1	0
RLU	105221	41255	26377	9073	3646	1749	1024	581
S/B	181	71	45	16	6	3	2	1
% Conversion	31	12	7	2	1	0.2	0.03	0

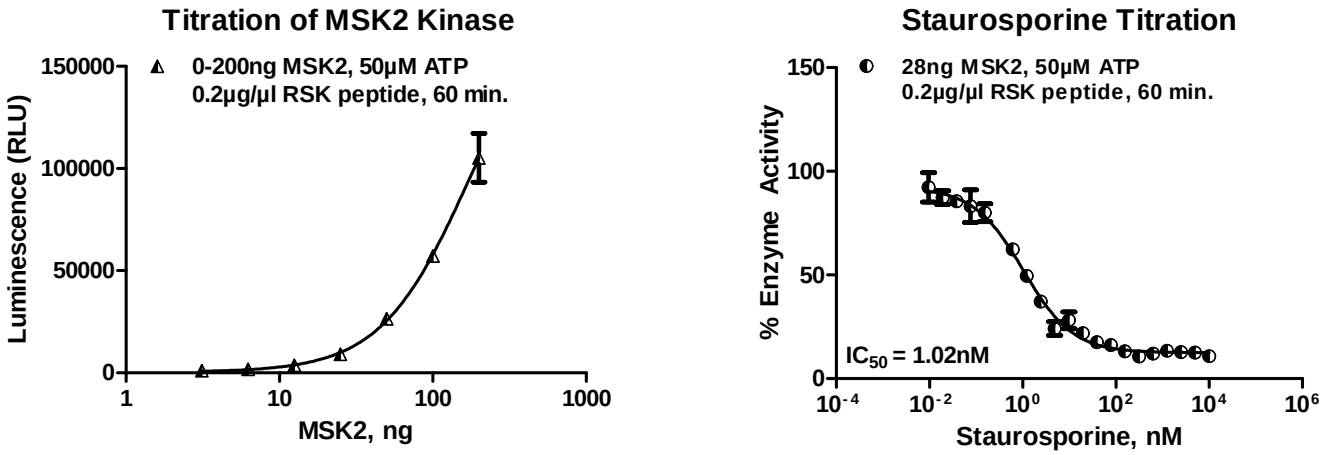

Figure 3. MSK2 Kinase Assay Development. (A) MSK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 28ng of MSK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
MSK2 Kinase Enzyme System	Promega	V5080
ADP-Glo™ + MSK2 Kinase Enzyme System	Promega	V5081

MSK2 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT