ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: MST1

MST1 Kinase Assay

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Scientific Background:

MST1 belongs to a family of proteins that share similarity with a budding yeast serine/threonine kinase, sterile-20 (Ste20). Endogenous full-length MST1 is activated by a variety of stressful stimuli, accompanied by the secondary appearance of a 36 kDa Thr183-phosphorylated, caspase-cleaved catalytic fragment (1). Recombinant MST1 undergo a robust autoactivation in vitro, mediated by an intramolecular autophosphorylation on the activation loop of an MST dimmer. MST1 can initiate apoptosis when transiently overexpressed in mammalian cells. Interference with the ability of endogenous MST1 to associate with the putative tumor suppressor proteins Nore1/RASSF can inhibit Ras-induced apoptosis (2).

1. De Souza, P M. et al: Mammalian Sterile20-like kinase 1 and the regulation of apoptosis. Biochem Soc Trans. 2004 Jun;32(Pt3):485-8.

2. Avruch, J. et al: Nore1 and RASSF1 Regulation of Cell Proliferation and of the MST1/2 Kinases. Methods Enzymol. 2005;407:290-310.

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.

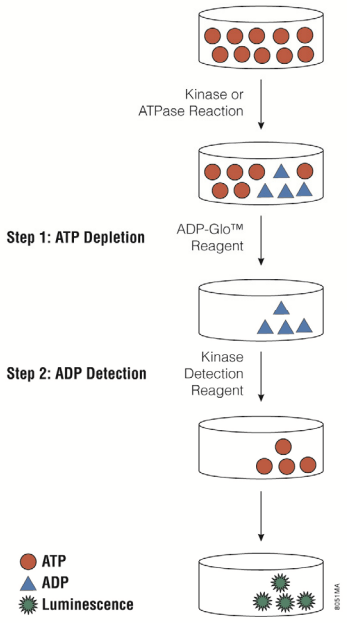

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

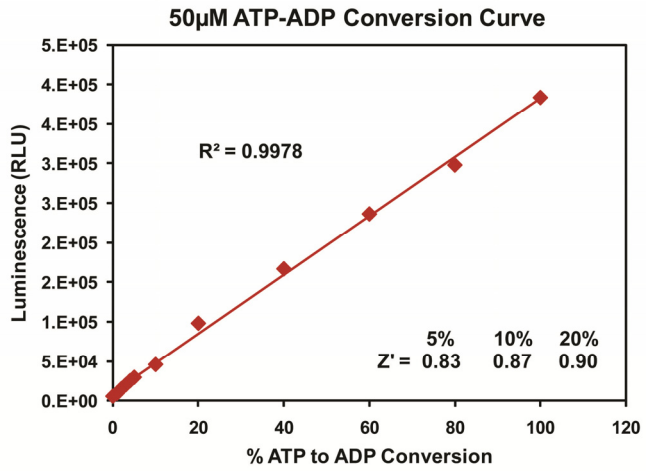

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, 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. MST1 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.

MST1, ng	200	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0
RLU	295279	270961	232784	186642	122071	77065	40872	22041	11411	5431	963
S/B	307	281	242	194	127	80	42	23	12	6	1
% Conversion	89	82	70	56	36	23	12	6	3	0.8	0

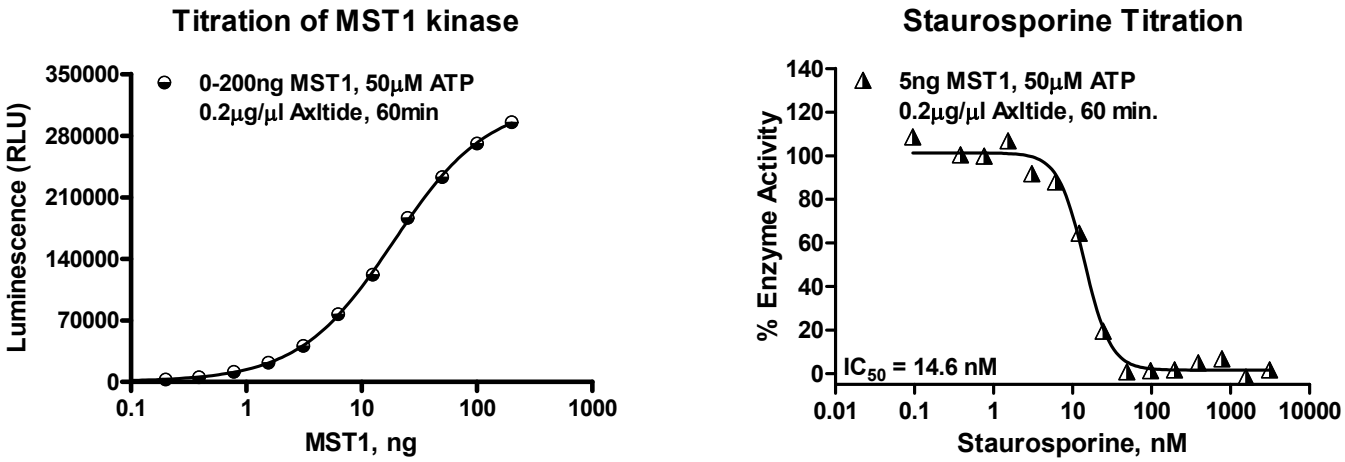

Figure 3. MST1 Kinase Assay Development. (A) MST1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 5ng of MST1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
MST1 Kinase Enzyme System	Promega	V4152
ADP-Glo™ + MST1 Kinase Enzyme System	Promega	V4153

MST1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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