ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: TAOK1

TAOK1 Kinase Assay

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Scientific Background:

TAOK1 is a serine/threonine-protein kinase involved in regulation of the p38-containing stress-responsive MAP kinase pathway and extracellular signal-regulated protein kinase (ERK) kinases (MEKs) (1). The activation of and binding to MEK3 by TAOK1 implicates TAOK1 in the regulation of the p38-containing stress-responsive MAP kinase pathway. A microtubule affinity-regulating kinase kinase, TAOK1 (also known as MARKK) is an important regulator of mitotic progression, required for both chromosome congression and checkpoint-induced anaphase delay (2).

1. Hutchison, M. et al: Isolation of TAO1, a protein kinase that activates MEKs in stress-activated protein kinase cascades. J. Biol. Chem. 1998; 273(44):28625-32.

2. Draviam, V.M. et al: A functional genomic screen identifies a role for TAO1 kinase in spindle-checkpoint signalling. Nat. Cell Biol. 2007; 9(5):556-64.

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.

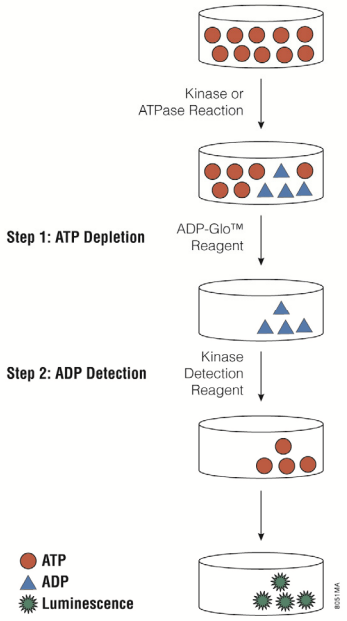

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

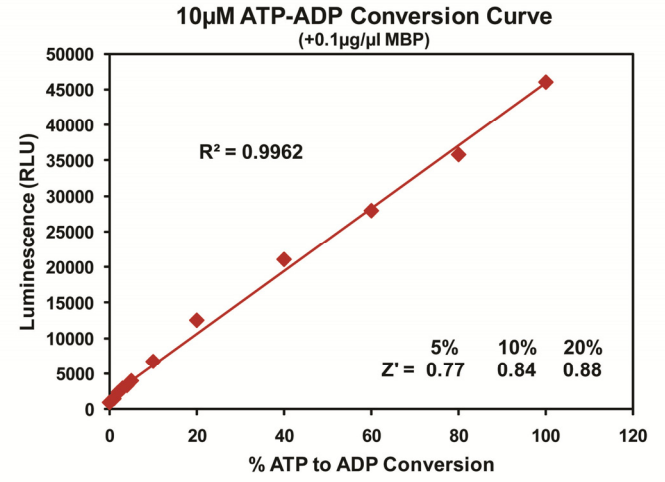

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. TAOK1 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.

TAOK1, ng	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	75756	75096	71579	63544	49349	36758	26292	16100	10005	5725	542
S/B	140	139	132	117	91	68	49	30	18	11	1
% Conversion	98	97	92	82	63	47	33	20	12	6	0

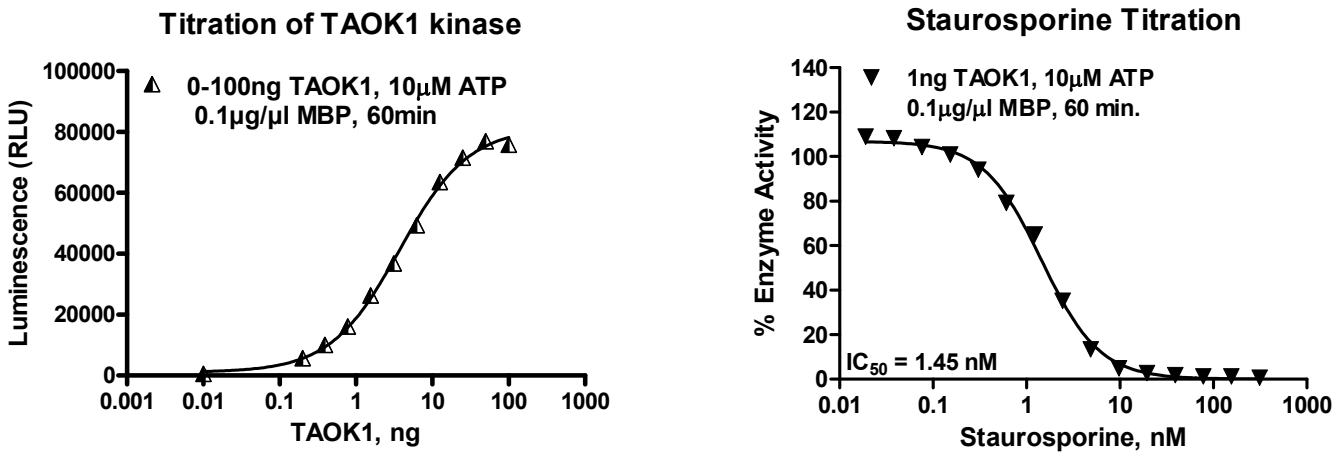

Figure 3. TAOK1 Kinase Assay Development. **(A) TAOK1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of TAOK1 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
TAOK1 Kinase Enzyme System	Promega	V4090
ADP-Glo™ + TAOK1 Kinase Enzyme System	Promega	V4091

TAOK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.