ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

TAOK2 Kinase Assay

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Scientific Background:

Thousand and one-amino acid protein kinase 2 (TAOK 2) is part of the stress-sensitive kinase cascade and activation of and binding to MKK3 by TAOK2 implicates it in the regulation of the p38-containing stress-responsive MAP kinase pathway (1). TAOK2 protein kinase activates p38 mitogen-activated protein kinase cascade in vitro and in cells by phosphorylating the MAP/ERK kinases MKK3 and MKK6. TAO2 may play an important role in the activation of specific intracellular signaling pathways that are unique to osmotic stress (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; 273:28625-32.

2. Chen, Z. et al: TAO (thousand-and-one amino acid) protein kinases mediate signaling from carbachol to p38 mitogen-activated protein kinase and ternary complex factors. J. Biol. Chem. 2003; 278: 22278-83.

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.

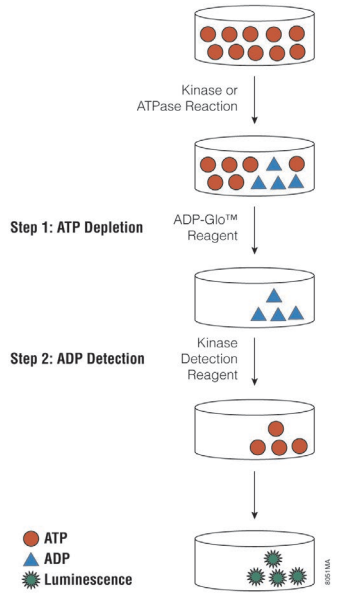

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

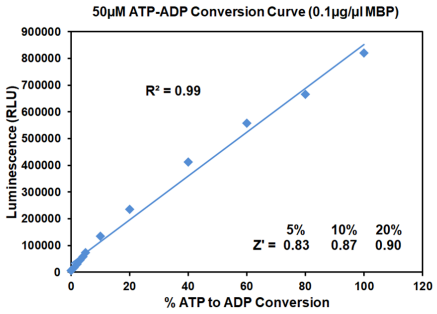

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0
Luminescence	297,226	139,473	73,830	42,197	20,853	10,659	6,216	4,253	2,807
S/B	106	50	26	15	7	4	2	2	1
% Conversion	34	15	7	3	1	0	0	0	0

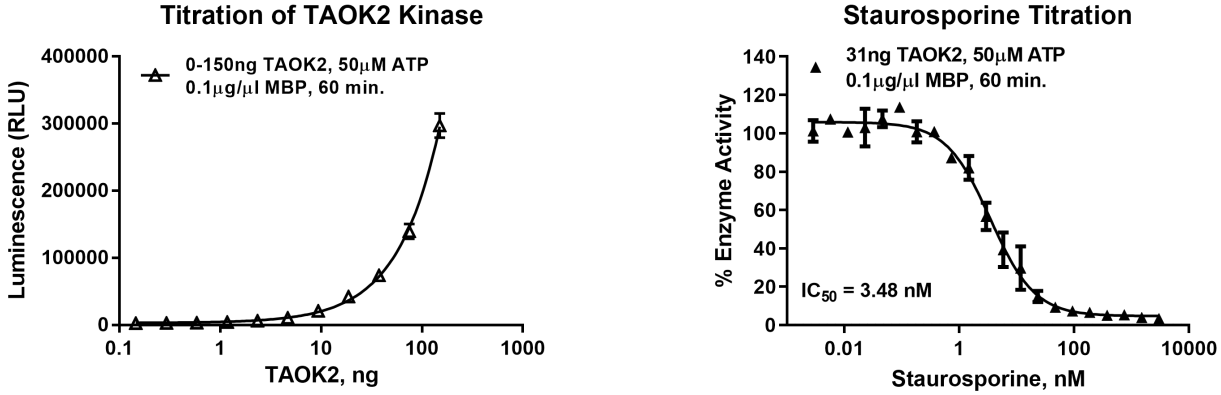

Figure 3. TAOK2 Kinase Assay Development. (A) TAOK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 31ng of TAOK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TAOK2 Kinase Enzyme System	10µg	VA7303
	1mg	VA7304
ADP-Glo™ + TAOK2 Kinase Enzyme System	1 Each	VA7305