ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: TOPK

TOPK Kinase Assay

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Scientific Background:

TOPK is a MAPK kinase that phosphorylates p38 MAPK and is activated in a cell-cycle-dependent manner in neuronal progenitor cells in vitro (1). Expression of TOPK is detected in male germ line progenitor cells, activated T-cells, and a variety of lymphomas and leukemias. In vitro studies have shown that activated TOPK phosphorylated p38MAPK but not JNK or ERK. TOPK activation requires phosphorylation by both the M-phase CDK1/CyclinB kinase complex and another unknown kinase, possibly RafC or RafA. TOPK may play an important role in linking extracellular signals to an intracellular state, possibly allowing extracellular influence on the cell-cycle-related processes of proliferation or differentiation (2).

1. Matsumoto, S. et al: Characterization of a MAPKK-like protein kinase TOPK. Biochem Biophys Res Commun. 2004; 325: 997-1004.

2. Simons-Evelyn, M. et al: PBK/TOPK is a novel mitotic kinase which is upregulated in Burkitt's lymphoma and other highly proliferative malignant cells. Blood Cells Mol Dis, 2001; 27: 825-829.

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.

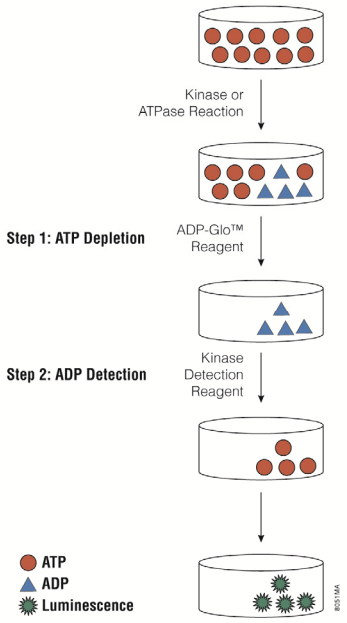

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

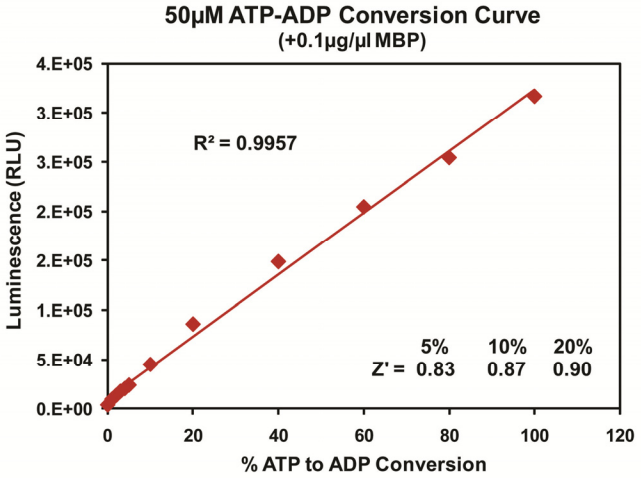

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. TOPK 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.

TOPK, ng	200	100	50	25	13	6.3	3.1	0
RLU	78318	34747	14281	8151	4060	2178	1434	991
S/B	79	35	14	8	4	2.2	1.4	1
% Conversion	31	13	5	2	1	0.4	0.3	0

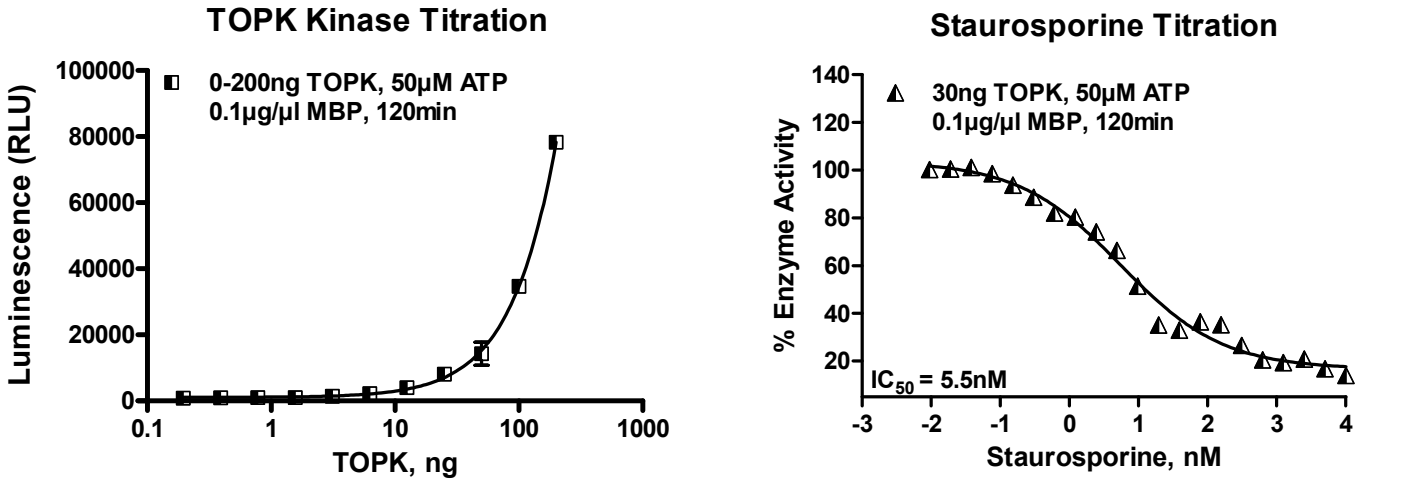

Figure 3. TOPK Kinase Assay Development. **(A) TOPK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 30ng of TOPK to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
TOPK Kinase Enzyme System	Promega	V4094
ADP-Glo™ + TOPK Kinase Enzyme System	Promega	V4095

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

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