TBK1 Kinase Assay

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Scientific Background:

TBK1, also known as NAK or NFkB-activating kinase, is an upstream protein kinase that can phosphorylate and activate the IkB kinases (1). Activation of IkB kinases allows the phosphorylation of IkB protein which is then degraded via the ubiquitination pathway. This mechanism allows the activation of the NFkB transcriptional complex. TBK1 is a specific upstream regulator of IkB kinases and can also interact and the IkB protein TANK. TBK1 is a component of the virus-activated kinase that phosphorylate IRF3 and IRF7 allowing their dimerization and translocation to the nucleus, where they induce transcription of interferon (2).

1. Tojima, Y. et al: NAK is an I-kappa-B kinase-activating kinase. Nature 404: 778-782, 2000.
2. Sharma, S. et al: Triggering the interferon antiviral response through an IKK-related pathway. Science 300: 1148-1151, 2003.

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.

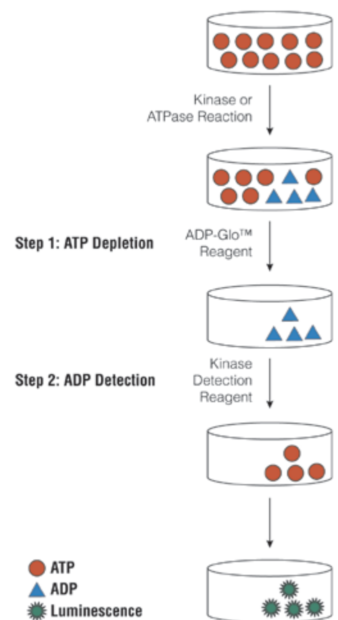

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

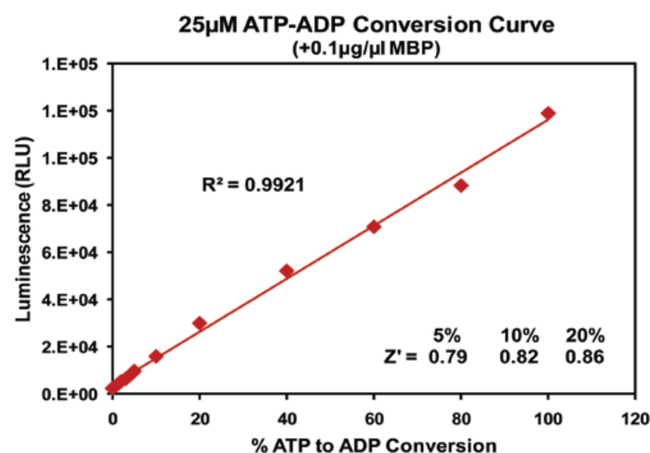

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. TBK1 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.

TBK1, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	106722	93831	82526	62920	42491	26759	16715	11818	8019	4992	2089
S/B	51	45	40	30	20	13	8	6	4	2	1
% Conversion	93.6	81.8	71.5	53.6	35.0	20.6	11.4	7.0	3.5	1.8	0

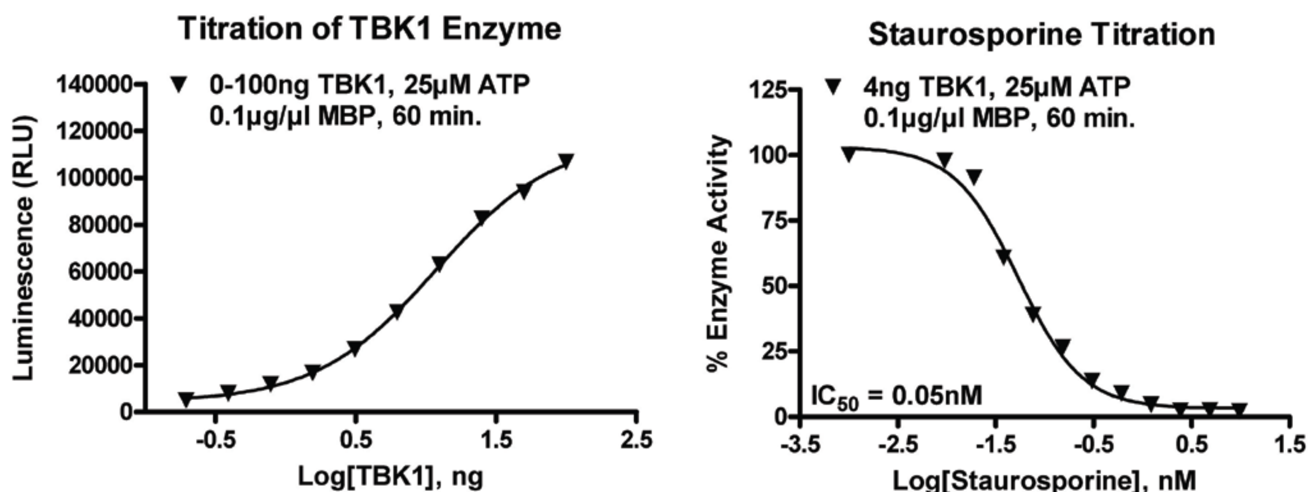

Figure 3. TBK1 Kinase Assay Development. (A) TBK1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 4ng of TBK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
TBK1 Kinase Enzyme System	Promega	V3991
ADP-Glo™ + TBK1 Kinase Enzyme System	Promega	V8291

TBK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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