TNIK Kinase Assay

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Scientific Background:

TNIK or TRAF2 and NCK interacting kinase is characterized by an N-terminal kinase domain and a C-terminal GCK domain that serves a regulatory function (1). TNIK is mainly expression in brain, heart, and spleen and it is a specific effector of RAP2 which regulate actin cytoskeleton (2). TNIK is autophosphorylated in a manner dependent upon lys54 in the ATP-binding pocket of its kinase domain and plays a main role in cytoskeleton regulation.

1. Fu, C. A. et.al: TNIK, a novel member of the germinal center kinase family that activates the c-Jun N-terminal kinase pathway and regulates the cytoskeleton. J. Biol. Chem. 274: 30729-30737, 1999.

2. Taira, K. et.al: The Traf2- and Nck-interacting kinase as a putative effector of Rap2 to regulate actin cytoskeleton. J. Biol. Chem. 279: 49488-49496, 2004.

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.

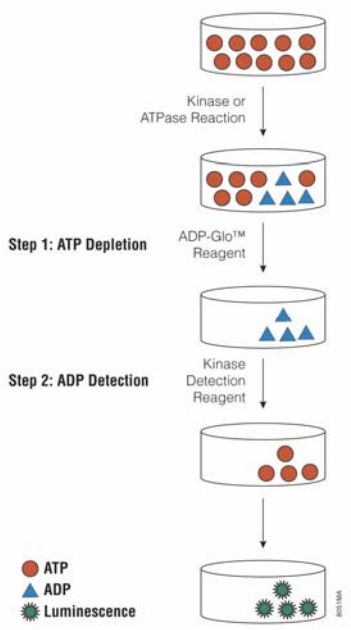


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

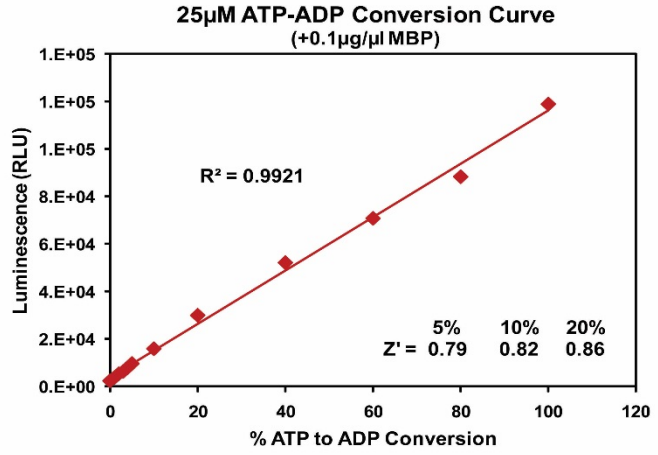


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

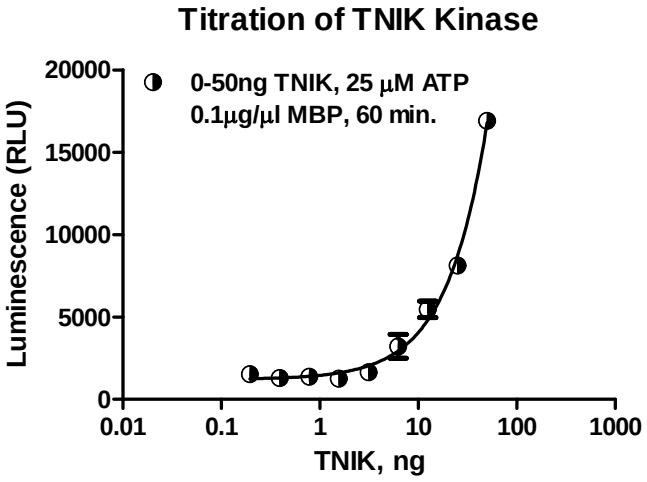
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. TNIK 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.

TNIK, ng	50	25	13	6.3	3.1	1.6	0
RLU	16903	8133	5452	3215	1644	1261	1083
S/B	16	8	5	3	1.5	1.2	1
% Conversion	9	4	2	1.20	0.31	0.10	0



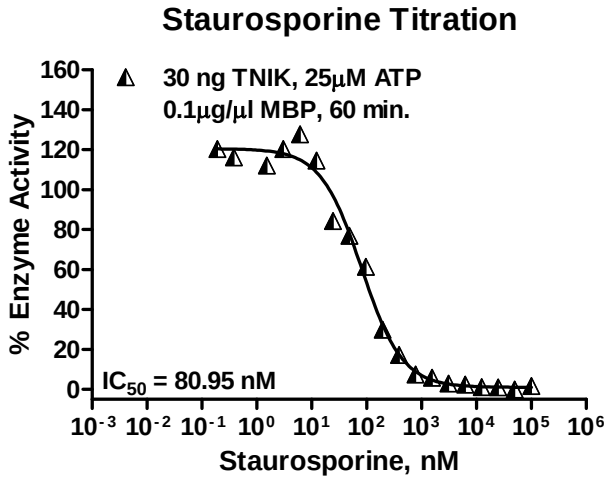


Figure 3. TNIK Kinase Assay Development. **(A) TNIK enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 30ng of TNIK to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
TNIK Kinase Enzyme System	Promega	V4158
ADP-Glo™ + TNIK Kinase Enzyme System	Promega	V4159

TNIK Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2.5mM MnCl2; 50µM DTT.