Description

The TNIK Kinase Assay Kit is designed to measure TNIK kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant TNIK kinase, kinase substrate, ATP and kinase assay buffer for 100 enzyme reactions.

Background

TNIK (TRAF2 and NCK interacting kinase) is characterized by an N-terminal kinase domain and a C-terminal GCK domain (Germinal Center Kinase domain) that serves a regulatory function (1). TNIK is mainly expressed in the brain, heart, and spleen. It is a specific effector of RAP2 (RAP2A, member of RAS oncogene family), which regulates the actin cytoskeleton (2). TNIK autophosphorylation depends upon Lys54 in the ATP-binding pocket of its kinase domain and plays a major role in cytoskeleton regulation.

Applications

- Study enzyme kinetics and screen small molecular inhibitors for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
79304	TNIK*	>1 µg**	-80°C
79334	Kinase assay buffer 1 (5x)	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
40535	Myelin basic protein (MBP), 5 mg/ml	100 µl	-20°C
79696	96-well plate, white	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube*
*** Excess material has been provided for ease of retrieval.*

Materials Required but Not Supplied

Name	Catalog #
Kinase-Glo MAX (Promega #V6071)	Promega #V6071
Microplate reader capable of reading luminescence	-
Adjustable micropipettor and sterile tips	-
30°C incubator	-

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle

Kinase activity is measured using **Kinase-Glo™ Max (Promega; #V6071)**. The addition of the reagent results in the generation of a luminescent signal that correlates with the amount of ATP. The reagent is linear to 100µM ATP.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw 5x Kinase assay buffer 1, ATP (500 µM), and Myelin basic protein (MBP) 5 mg/ml.
2. Prepare the Master Mix (25 µl per well): N wells x (5 µl Kinase assay buffer 1 (5x) + 1 µl ATP (500 µM) + 1 µl MBP (5 mg/ml) + 18 µl distilled water). Add 25 µl to every well.
3. Prepare 3 ml of 1x Kinase assay buffer by mixing 600 µl of 5x Kinase assay buffer with 2400 µl distilled water. Three ml of 1x Kinase assay buffer is sufficient for 100 reactions.
4. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Kinase Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Kinase Assay Buffer (Diluent Solution).

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO should not exceed 1%.
5. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor.” For the “Positive Control" and “Blank,” add Diluent Solution (either distilled water or 10% DMSO in water, as described above).
6. To the wells designated as "Blank", add 20 µl of **1x Kinase assay buffer**.
7. Thaw TNIK on ice. Briefly spin the tube to recover its full contents. Dilute the protein kinase to 0.6 ng/µl using 1x Kinase assay buffer.

   Notes: the concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.

Calculate the amount of protein kinase required for the assay and dilute enough for the assay. Aliquot unused protein into 2-4 aliquots as may be necessary (single use aliquots) and store them at -80°C. Avoid multiple freeze/thaw cycles. Do not re-use the aliquots more than once or twice and do not re-use the diluted kinase.

8. Initiate the reaction by adding 20 µl of diluted TNIK to the wells designated “Positive Control” and "Test Inhibitor".

Component	Blank	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x Kinase Buffer	20 µl	-	-
TNIK (0.6 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

9. Incubate at 30˚C for 45 minutes.
10. During the incubation, thaw the Kinase-Glo Max reagent. At the end of the 45-minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover the plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
11. Immediately read in a luminometer or a microplate reader capable of reading luminescence. The “Blank” value is subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

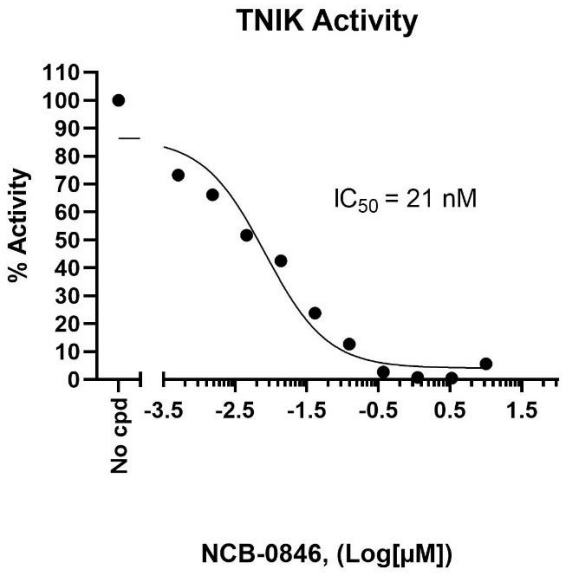

Figure 1: Inhibition of TNIK kinase Activity by NCB-0845. The inhibition of TNIK kinase activity by NCB-0846 was measured in the presence of increasing inhibitor concentrations using the TNIK Kinase Assay Kit (BPS Bioscience #78370). The Blank value was subtracted from all other values. Results are expressed as percent of control (kinase activity in the absence of inhibitor, set at 100%).

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

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.

Related Products

Products	*Catalog #*	*Size*
TNIK, GST-Tag	79304	10 µg
MBP, His-Tag	40535	100 µg
Kinase Buffer 1	79334	10 ml
ATP (500 µM)	79686	200 µl