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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Chemi-Verse™ TNK1 Kinase Assay Kit

#82159
96 reactions

Description
The Chemi-Verse™ TNK1 Kinase Assay Kit is designed to measure TNK1 (tyrosine kinase non receptor 1) tyrosine kinase activity for screening and profiling applications using ADP-Glo™ as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant TNK1 kinase (amino acids 106-390), kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background
TNK1, also known as tyrosine kinase non receptor 1 or thirty-eight-negative kinase -1, is part of the NRTK family (non-receptor tyrosine kinases), in particular the ACK sub-family. This sub-family is composed of two members, TNK1 and TNK2, and they are unique in that they have a putative ubiquitin association domain (UBA). TNK1 has a tissue-restricted expression profile in adults, being present in prostate, testis, ovaries, colon, and the small intestine. During development it is more widely expressed, and it is involved in hematopoiesis. TNK1 is involved in the primary lymphoid malignancies by mediating cell survival, and it has been shown to sensitize cancer cells to stress by reducing phosphorylation of STAT3 (signal transducer and activator of transcription 3) and STAT5 in L540 cells. TNK1 is inhibited by interacting with the protein 14-3-3, a binding that involves MARK (MAP/microtubule affinity-regulating kinase)-mediated phosphorylation. Regulation of its activity also involves ubiquitin. When active, TNK1 can induce growth-factor independent lymphoid cell proliferation *in vitro*. The use of an inhibitor, TP-5801, has shown efficacy in suppressing tumor growth *in vitro* and *in vivo*. The deeper understanding of the mode of action of this unique kinase and of the effects of its inhibition may open new therapeutic avenues.

Applications
Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
80019	TNK1, GST-Th-Tag*	10 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	50 µl	-20°C
	CSKtide (1 mg/ml)	500 µl	-20°C
79696	White 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Name	Ordering Information
ADP-Glo™ Kinase Assay	Promega #V6930
DTT (Dithiothreitol), 1M, optional	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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

The **ADP-Glo™ Kinase Assay (Promega #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear to 1 mM 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.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

1. Thaw **5x Kinase Assay Buffer 1**, **500 µM ATP,** and **CSKtide (1 mg/ml).**

   *Optional: If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.*

2. Prepare 3 ml of **1x Kinase Assay Buffer 1** by mixing 600 µl of **5x Kinase Assay Buffer 1** with 2,400 µl of distilled water.

   *Note: Three (3 ml) of **1x Kinase Assay Buffer 1** is sufficient for 100 reactions.*

3. Prepare a **Master Mix** (12.5 µl/well): N wells x (6 µl of 5x Kinase Assay Buffer 1 + 0.5 µl of 500 µM ATP + 5 µl of CSKtide (1 mg/ml) + 1 µl of distilled water).

4. Add 12.5 µl of Master Mix to every well.

5. Prepare the **Test Inhibitor** (2.5 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 25 µl.

   5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in **1x Kinase Assay Buffer 1**, 10-fold more concentrated than the desired final concentrations.

For the positive and negative controls, use **1x Kinase Assay Buffer 1** (Diluent Solution).

OR

5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in **1x Kinase Assay Buffer 1** 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 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

6. Add 2.5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.
7. Add 2.5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.
8. Add 10 µl of 1x Kinase Assay Buffer 1 to the "Blank" wells.
9. Thaw **TNK1** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10 µl/well) to 10 ng/µl with 1x Kinase Assay Buffer 1.
11. Initiate the reaction by adding 10 µl of diluted kinase to the wells designated “Positive Control” and "Test Inhibitor".
12. Incubate at 30°C for 45 minutes.
13. Thaw the ADP-Glo™ reagent.
14. At the end of the 45-minute reaction, add 25 µl of ADP-Glo™ reagent to each well.
15. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.
16. Thaw the Kinase Detection Reagent.
17. Add 50 µl of Kinase Detection reagent to each well.
18. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.
19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.
20. The “Blank” value should be subtracted from all other readings.

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Assay Buffer 1	10 µl	-	-
Diluted TNK1 (10 ng/µl)	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

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

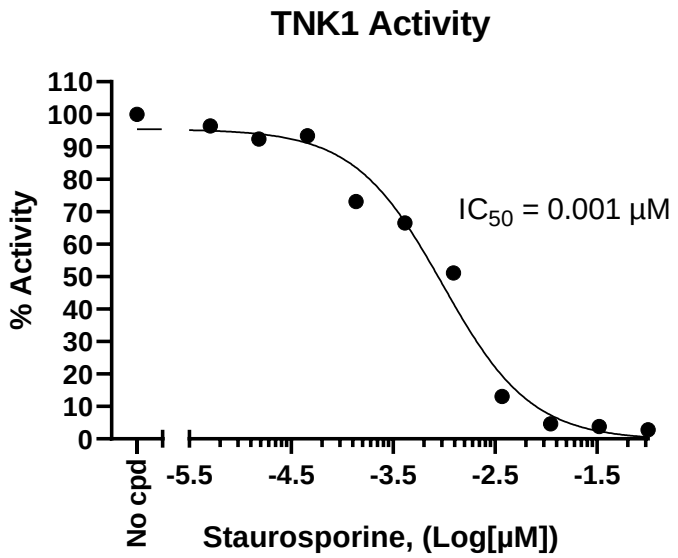

Figure 1: Inhibition of TNK1 kinase activity by Staurosporine.
The inhibition of TNK1 kinase activity was measured in the presence of increasing concentrations of Staurosporine (SelleckChem #S1421). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data shown is representative. 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

Chan T., *et al.*, 2021 *Nature Communications* 12: 5337.

Related Products

Products	*Catalog #*	*Size*
MARK1, GST-Tag Recombinant	40119	10 µg
MARK3, GST-Tag Recombinant	40120	10 µg
STAT3 Luciferase Reporter THP-1 Cell Line	78498	2 vials
STAT3 Reporter Jurkat Cell Line	78497	2 vials
STAT5 Luciferase Reporter Ba/F3 Cell Line	79772	2 vials