

TNK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # T12-11G

Lot # W3254-6

Product Description

Recombinant human TNK1 (1-510) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_003985](#).

Gene Aliases

MGC46193

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

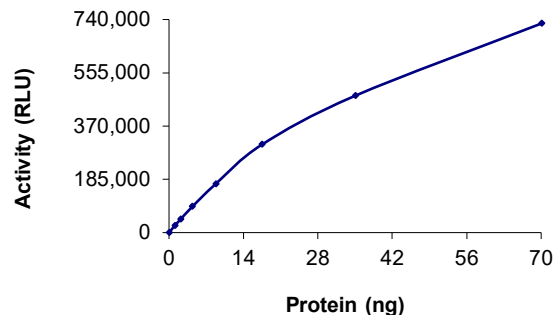
Scientific Background

TNK1 or tyrosine kinase, non-receptor 1 belongs to the tyrosine protein kinase family which are important regulators of intracellular signal transduction pathways mediating cellular proliferation, survival, and development. TNK1 is highly expressed in fetal tissues and at lower levels in few adult tissues. TNK1 may function in signaling pathways utilized broadly during fetal development, and more selectively in adult tissues. TNK1 plays a negative regulatory role in the Ras-Raf1-MAPK pathway, and knockout mice have been shown to develop spontaneous tumors, suggesting a role as a tumor suppressor gene (1).

References

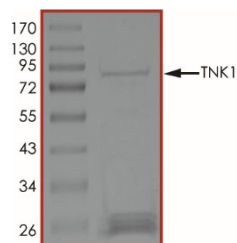
1. Hoehn, G. T. et.al: Tnk1: a novel intracellular tyrosine kinase gene isolated from human umbilical cord blood CD34(+)/Lin(-)/CD38(+) stem/progenitor cells. Oncogene 12: 903-913, 1996.

Specific Activity



The specific activity of TNK1 was determined to be **38 nmol/min/mg** as per activity assay protocol, and was equivalent to **15 nmol/min/mg** as per radiometric assay.

Purity



The purity of TNK1 was determined to be **>70%** by densitometry. TNK1 Approx. MW **85kDa**.

TNK1, Active

Catalog #	T12-11G
Specific Activity	38 nmol/min/mg
Lot #	W3254-6
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

"THIS PRODUCT SHALL NOT BE USED TO COMMERCIALY SCREEN DRUG MOLECULES DEVELOPED AS TNK1 OR TNK1 INHIBITORS. ANY SUCH ACTIVITY WILL BE OUTSIDE THE SCOPE OF THE RESEARCH USE ONLY LABEL LICENCE" THE PRODUCT IS PROTECTED BY THE FOLLOWING PATENTS: US5,910,426; US5,852,184; US5,821,069; US5,716,818,US5,658,791

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: T12-11G)

Active TNK1 (0.05 μ g/ μ l) diluted with Kinase Dilution Buffer IX (1x) (Catalog #: K29-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of active TNK1 for optimal results).

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl₂ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250 μ M.

Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50 μ M.

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Substrate (Catalog #: M42-54G)

Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 0.2mg/ml.

Assay Protocol

The TNK1 assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the TNK1 reaction. The ADP-Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

- Step 1.** Thaw the Active TNK1, Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 μ L enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1x), in a pre-chilled 96-well plate.
- Step 2.** Prepare a substrate/ATP mixture as follows (25 μ M example):

Component	Amount (μ L)	Component	Amount (μ L)
10mM ATP Solution	1	Substrate at 0.2 mg/mL	80
Kinase Assay Buffer III (5x)	79		

- Step 3.** Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5 μ L:

Component 1.	3 μ l of diluted Active TNK1 (Catalog # T12-11G).
Component 2.	2 μ l of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

- Step 4.** Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1x).
- Step 5.** Incubate at ambient temperature for 40 minutes.
- Step 6.** After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 μ l of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
- Step 7.** Then add 10 μ l of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
- Step 8.** Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).
- Step 9.** Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/ μ g or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

THIS PRODUCT SHALL NOT BE USED TO COMMERCIALY SCREEN DRUG MOLECULES DEVELOPED AS TNK1 OR TNK1 INHIBITORS. ANY SUCH ACTIVITY WILL BE OUTSIDE THE SCOPE OF THE RESEARCH USE ONLY LABEL LICENCE" THE PRODUCT IS PROTECTED BY THE FOLLOWING PATENTS: US5,910,426; US5,852,184; US5,821,069; US5,716,818,US5,658,791

MATERIAL SAFETY DATA SHEET

Article 1 - Product Identification and Use

Product Name: TNK1, Active

Catalog # T12-11G

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name: SignalChem Pharmaceuticals Inc.
Street Address: 110-13120 Vanier Place
City, Prov. Postal Code: Richmond, BC, V6V 2J2
Fax: 604-232-4601
EMERGENCY PHONE: 604-232-4600

Article 2 - Hazardous Ingredients

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

Article 3 - Physical Data

This product consists of purified protein in Tris-HCl buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

Article 4 - Fire and Explosion Hazard

NOT APPLICABLE

Article 5 - Reactivity Data

NOT APPLICABLE

Article 6 - Toxicologically Data

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

Article 7 - Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

*****MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES*****

- Wear protective equipment.
 - Absorb on sand or vermiculite and place in closed containers for disposal.
 - Observe all federal, state and local environmental regulations.
-

Article 8 - First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
 - In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
 - If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
 - In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.
-

Article 9 - Preparation

Prepared by: Mya Zhang

Phone#: 1-866-954-6273

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

"THIS PRODUCT SHALL NOT BE USED TO COMMERCIALY SCREEN DRUG MOLECULES DEVELOPED AS TNK1 OR TNK1 INHIBITORS. ANY SUCH ACTIVITY WILL BE OUTSIDE THE SCOPE OF THE RESEARCH USE ONLY LABEL LICENCE" THE PRODUCT IS PROTECTED BY THE FOLLOWING PATENTS: US5,910,426; US5,852,184; US5,821,069; US5,716,818,US5,658,791