

TNNI3K, Active

Recombinant human protein expressed in Sf9 cells

Catalog # T13-11G

Lot # H2969-15

Product Description

Recombinant human TNNI3K (300-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC117262](#).

Gene Aliases

CARK, CCDD

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

The Cardiac Troponin I - interacting kinase (TNNI3K) is a serine/threonine-protein kinase, that is a member of the MAPKKK family (1). As a cardiac-specific kinase, it contains Ankyrin repeat, protein kinase, and serine-rich domains and highly expressed in the cardiomyocytes (1). Overexpression of TNNI3K has been shown to increase the progression of cardiomyopathy in mice through the Tyr and Ser/Thr kinase activity (2). The TNNI3K mutation has been involved in a familial syndrome of conduction system disease and dilated cardiomyopathy by causing the aggregation of this protein (3).

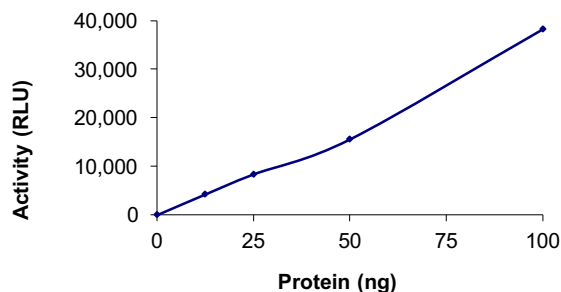
References

1. Y. Zhao, et al: Cloning and characterization of a novel cardiac-specific kinase that interacts specifically with cardiac troponin I. *J Mol Med.* 81: 297-304, 2003.
2. H. Tang, et al: Overexpression of TNNI3K, a cardiac specific MAPKKK, promotes cardiac dysfunction. *J Mol Cell Cardiol.* 54: 101-111, 2013.
3. J. L. Theis, et al: TNNI3K mutation in familial syndrome of conduction system disease, atrial tachyarrhythmia and dilated cardiomyopathy. *Human Molecular Genetics.* 23: 5793-5804, 2014.

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

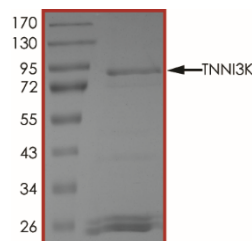
FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

Specific Activity



The specific activity of TNNI3K was determined to be **2.6 nmol /min/mg** as per activity assay protocol.

Purity



The purity of TNNI3K was determined to be **>75%** by densitometry, approx. MW **~87kDa**.

TNNI3K, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	T13-11G
Specific Activity	2.6 nmol/min/mg
Lot #	H2969-15
Purity	>75%
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.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: T13-11G)

Active TNNI3K (0.05 µg/µl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) 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 IRAK1 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 250µM.

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 50µM.

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

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

Substrate (Catalog #: C10-58)

CHKtide synthetic peptide substrate (KKKVSRSGLYRSPSPENLNRP) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

The TNNI3K assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the TNNI3K 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 TNNI3K, Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 µL 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 µM example):

Component	Amount (µL)	Component	Amount (µL)
10µM ATP Solution	1	Substrate at 1mg/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 5µL:

Component 1.	3µl of diluted Active TNNI3K (Catalog # T13-11G).
Component 2.	2µl 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 5µl 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 10µl 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/µg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in µg 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.