TNK1, Active
Recombinant human protein expressed in Sf9 cells
Catalog # T12-11G
Lot # M2672-6

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

Specific Activity
The specific activity of TNK1 was determined to be 34.2 nmol/min/mg as per activity assay protocol, and was equivalent to 14.3 nmol/min/mg as per radiometric assay.

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

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 COMMERCIALLY 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 (0.05µg/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 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 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-GloTM 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 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):

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10µM ATP Solution</td>
<td>1</td>
</tr>
<tr>
<td>Kinase Assay Buffer III (5x)</td>
<td>79</td>
</tr>
<tr>
<td>Substrate at 1mg/mL</td>
<td>80</td>
</tr>
</tbody>
</table>

**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 TNK1 (Catalog # T12-11G).
- 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.