

## TPM3-ROS1, Active

Human recombinant fusion protein expressed in Sf9 cells

**Catalog # R14-19CG**

Lot # G1475-2

### Product Description

Recombinant human fusion protein TPM3 (1-258)-ROS1 (1881-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The TPM3 gene accession number is [NM\\_152263](#) and ROS1's one is [NM\\_002944](#).

### Gene Aliases

TPM3: CAPM1; CFTD; hscp30; NEM1; OK/SW-cl.5; TM-5; TM3; TM30; TM30nm; TM5; TPMsk3; TRK  
ROS1: c-ros-1; MCF3; ROS

### Formulation

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

### Storage and Stability

Store product at  $-70^{\circ}\text{C}$ . 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.

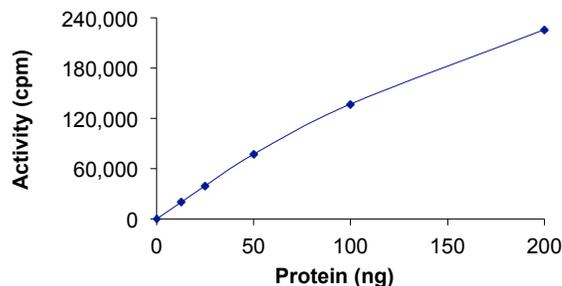
### Scientific Background

TPM3 is a member of the tropomyosin family of actin-binding proteins which are dimers of coiled-coil proteins that provide stability to actin filaments and regulate access of other actin-binding proteins. Mutations in TPM3 gene result in autosomal dominant nemaline myopathy and other muscle disorders (1). TPM3 gene locus is involved in translocations with other loci including c-ros oncogene 1 (ROS1) which result in the formation of the TPM3-ROS1 fusion protein that act as an oncogene. TPM3-ROS1 fusion gene product has been detected in lung adenocarcinoma is higher in lung adenocarcinoma with wild-type EGFR (2).

### References

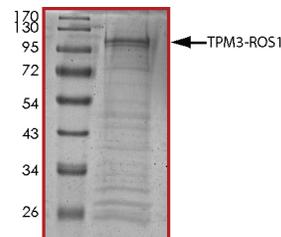
1. Lawlor, M W. et al: Mutations of tropomyosin 3 (TPM3) are common and associated with type 1 myofiber hypotrophy in ongenital fiber type disproportion. Hum Mutat. 2010 Feb;31(2):176-83.
2. Zhao C. et al: Detecting ALK, ROS1 and RET Fusion Genes in Cell Block Samples. Transl Oncol. 2014 Jun 17;7 (3):363-7.

### Specific Activity



The specific activity of TPM3-ROS1 was determined to be **95 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of TPM3-ROS1 was determined to be **>70%** by densitometry, approx. MW **108kDa**.

## TPM3-ROS1, Active

Human recombinant fusion protein expressed in Sf9 cells

Catalog #	R14-19CG
Specific Activity	95 nmol/min/mg
Lot #	G1475-2
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr at $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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](mailto:orders@signalchem.com)  
[www.signalchem.com](http://www.signalchem.com)

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

# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: R14-19CG)

Active TPM3-ROS1 (0.05µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 TPM3-ROS1 for optimal results).

### Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

### Kinase Assay Buffer (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: I15-58)

IGF1Rtide synthetic peptide substrate (KKKSPGEYVNIEFG) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active TPM3-ROS1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1.** 10µl of diluted Active TPM3-ROS1 (Catalog # R14-19CG)
  - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog #I15-58)
  - Component 3.** 5µl distilled H<sub>2</sub>O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity (cpm) on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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

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