

## MSSK1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # S41-10G

Lot # Q054-1

### Product Description

Recombinant full-length human MSSK1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC117124](#).

### Gene Aliases

STK23, SRPK3, MGC102944

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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.

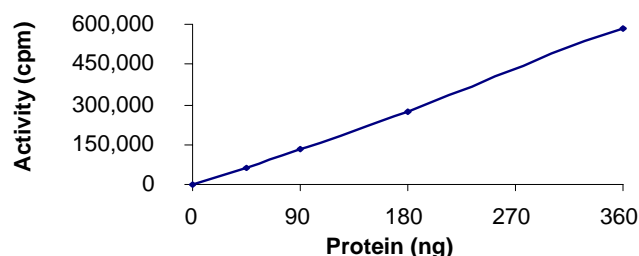
### Scientific Background

MSSK1, also known as SRPK3, is a muscle-specific protein kinase belonging to the serine arginine protein kinase family which phosphorylates serine/arginine repeat-containing proteins. Heart and skeletal muscle show high expression of the MSSK1/SRPK3 gene product. SRPK3-null mice display a new entity of type 2 fiber-specific myopathy with a marked increase in centrally placed nuclei. Transgenic mice overexpressing SRPK3 in skeletal muscle show severe myofiber degeneration and early lethality (1).

### References

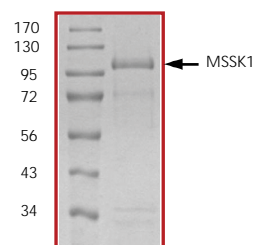
1. Nakagawa, O. et al: Centronuclear myopathy in mice lacking a novel muscle-specific protein kinase transcriptionally regulated by MEF2. *Genes Dev.* 2005 Sep 1;19(17):2066-77.

### Specific Activity



The specific activity of MSSK1 was determined to be **235 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of MSSK1 was determined to be **>80%** by densitometry, approx. MW **98kDa**.

## MSSK1, Active

Full-length recombinant protein expressed in Sf9 cells

|                     |  |
|---------------------|--|
| Catalog Number      | S41-10G  |
| Specific Activity   | 235 nmol/min/mg  |
| Specific Lot Number | Q054-1   |
| Purity              | >80%   |
| Concentration       | 0.1 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 #: S41-10G)

Active MSSK1 (0.1µg/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 MSSK1 for optimal results).

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

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

### Kinase Assay Buffer I (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 #: P15-58)

PKCtide peptide substrate (ERMRRPRKRGSVRRRV) 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 MSSK1, 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 MSSK1 (Catalog #S41-10G)
  - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #P15-58)
  - 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 5 µl [<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 20 µl 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 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.**