SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
M53-11G-05	5 µg
M53-11G-10	10 µg

MINK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # M53-11G

Lot # B1965-7

Product Description

Recombinant human MINK1 (1-320) was expressed by baculovirus in Sf9 cells using an N-terminal GST tag. The gene accession number is NM_015716.

Gene Aliases

B55; ZC3; MINK; YSK2; hMINK; MAP4K6; MGC21111; hMINKbeta

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.

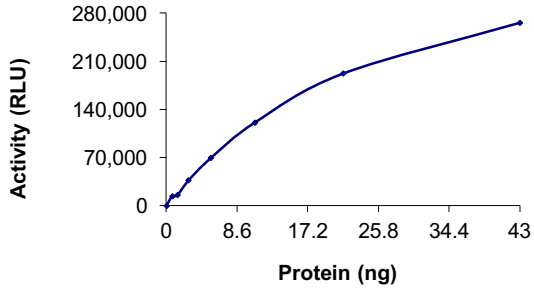
Scientific Background

MINK1 is a member of the germinal center family of kinases that are homologous to the Ste20 family and regulate a wide variety of cellular processes, including cell morphology, cytoskeletal rearrangement, and survival (1). Overexpression of kinase-dead mutants of MINK1 leads to enhanced cell spreading, actin stress fiber formation, adhesion to extracellular matrix and decreased cell motility and invasion. MINK is activated after Ras induction via a mechanism involving reactive oxygen species and mediates stimulation of p38 MAPK downstream of the Raf/ERK pathway (2).

References

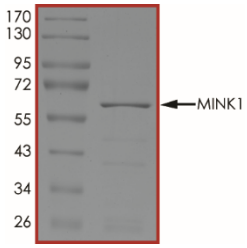
1. Hu, Y. et al: Identification and functional characterization of a novel human misshapen/Nck interacting kinase-related kinase, hMINK beta. J Biol Chem. 2004 Dec 24;279(52):54387-97.
2. Nicke, B. et al: Involvement of MINK, a Ste20 family kinase, in Ras oncogene-induced growth arrest in human ovarian surface epithelial cells. Mol Cell. 2005 Dec 9;20(5):673-85.

Specific Activity



The specific activity of MINK1 was determined to be **22 nmol /min/mg** as per activity assay protocol, and was equivalent to **22 nmol/min/mg** as per radiometric assay.

Purity



The purity was determined to be **>70%** by densitometry.
Approx. MW **~61kDa**.

MINK1, Active

Recombinant protein expressed in Sf9 cells

Catalog #	M53-11G
Specific Activity	22 nmol/min/mg
Lot #	B1965-7
Purity	>70%
Concentration	0.1 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

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M53-11G)

Active MINK1 (0.1μg/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 MINK1 for optimal results).

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

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ 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 #: M42-51N)

Myelin Basic Protein (MBP) substrate diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

The MINK1 assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the MINK1 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 MINK1, 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 MINK1 (Catalog # M53-11G).
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