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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
M19-11G-05	5 µg
M19-11G-10	10 µg

MLK3, Active

Recombinant human protein expressed in Sf9 cells

Catalog # M19-11G
Lot # L1933-7

Product Description

Recombinant human MLK3 (1-488) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The MLK3 gene accession number is NM_002419.

Gene Aliases

MAP3K11, PTK1, SPRK, MLK3, MGC17114, MLK-3

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°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.

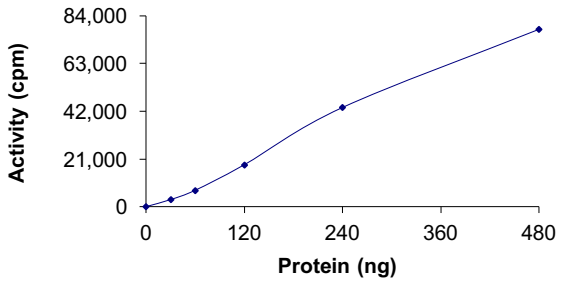
Scientific Background

MLK3 or Mixed-Lineage Kinase 3 is a mitogen-activated protein kinase kinase kinase capable of preferentially activating MAPK8/JNK kinase and functions as a positive regulator of JNK signaling pathway (1). MLK3 can directly phosphorylate and activate IkappaB kinase α and β, and is found to be involved in the transcription activity of NF-kappaB mediated by Rho family GTPases and CDC42. MLK3 is a signal-integrating kinase with conventional MAP3K catalytic activity and additional noncatalytic functions that contribute to RAF/ERK signalling (2). MLK3 is a component of the BRAF/RAF1 complex and is required for integrity of the complex and for activation of ERK by the complex.

References

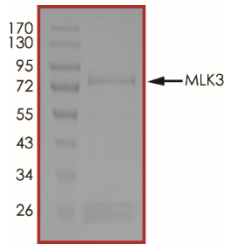
1. Ing, Y. L. et al: MLK-3: identification of a widely-expressed protein kinase bearing an SH3 domain and a leucine zipper-basic region domain. Oncogene 9: 1745-1750, 1994.
2. Chadee, D. N. et al: MLK3 is required for mitogen activation of B-Raf, ERK and cell proliferation. Nature Cell Biol. 6: 770-776,2004.

Specific Activity



The specific activity of MLK3 was determined to be **9 nmol /min/mg** as per activity assay protocol.

Purity



The purity of MLK3 was determined to be **>70%** by densitometry, approx. MW **~83kDa**.

MLK3, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	M19-11G
Specific Activity	9 nmol/min/mg
Lot #	L1933-7
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.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M19-11G)

Active MLK3 (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 MLK3 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 50ng/μl BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}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 [^{33}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 #: M42-51N)

MBP protein diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active MLK3, 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 MLK3 (Catalog # M19-11G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog # M42-51N)
- **Component 3.** 5μl distilled H_2O (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_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}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_2O) 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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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