

Certificate of Analysis

MLK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-039, 16-039-K, 16-039M

Parent Lot # D17DP011N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal GST-tagged, recombinant, human MLK3 amino acids 1-468 expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.

Purity 90% by SDS-PAGE and Coomassie blue staining. MW = 79kDa.

Specific Activity (Parent lot# D17DP011N): 62U/mg, where one unit of MLK3 activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.76mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzimidazole, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

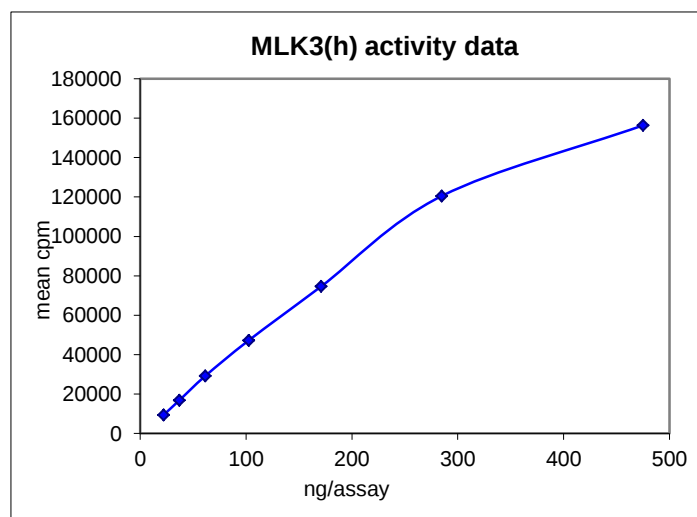
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

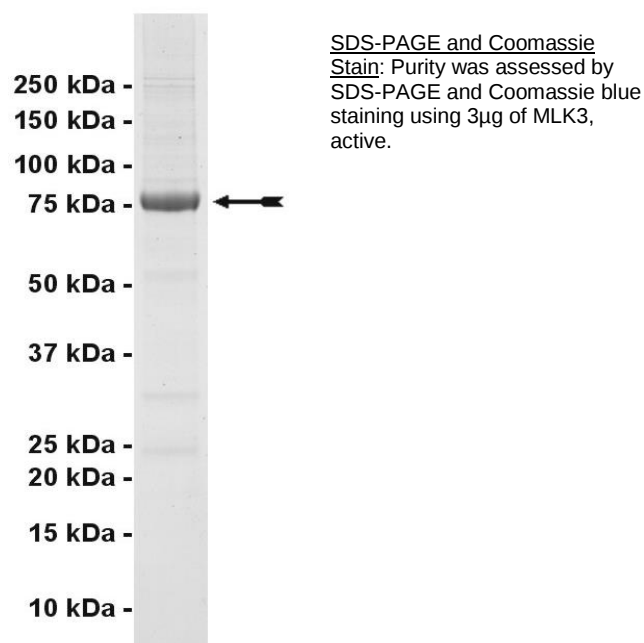
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 22–285ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as MLK3 with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Myelin basic protein:** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
3. **DTT:** Use at a final assay concentration of 5mM. Prepare a 1M stock and use 0.125µl per well.
4. **MLK3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 22–285ng per assay point.
5. **[γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 1500 - 2400cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of myelin basic protein to wells.
3. Add 0.125µl of 1M DTT to wells
4. Add **2.5µl (22–285ng) MLK3, active.**
5. Add 4.875µl of dH₂O.
6. Add 10µl of diluted [γ -³³P]ATP mixture.
7. Incubate for 30 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

Certificate of Analysis

MLK3, active Sequence Information

Protein	Human MLK3
Tags	N-terminal GST
Native sequence	M230 of the recombinant protein is equivalent to M1 of human MLK3
Accession number	GenBank NM_002419.2

Recombinant MLK3 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID   KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSKEFM  EPLKSLFLKS
241 PLGSWNGSGS  GGGGGGGGGR  PEGSPKAAGY  ANPVWTALFD  YEPGQDELA   LRKGDREVL
301 SRDAAISGDE  GWWAGQVGQ   VGIFPSNYVS  RGGGPPPEV   ASFQELRLEE  VIGIGGFGKV
361 YRGSWRGELV  AVKAARQDPD  EDISVTAESV  RQEARLFAML  AHPNIIALKA  VCLEEPNLCL
421 VMEYAAGGPL  SRALAGRRVP  PHVLVNWAVQ  IARGMHYLC   EALVPVIHRD  LKSNNILLQ
481 PIESDDMEHK  TLKITDFGLA  REWHKTTQMS  AAGTYAWMAP  EVIKASTFSK  GSDVWSFGVL
541 LWELLTGEVP  YRGIDCLAVA  YGVAVNKLTL  PIPSTCPEPF  AQLMADCWAQ  DPHRRPDFAS
601 ILQQLEALEA  QVLRMPRDS   FHSMQEGWKR  EIQGLFDELR  AKEKELLSRE  EELTRAAREQ
661 RSQAEQLRRR  EHLLAQWELE  VFERELTLL  QQVDRER

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Recombinant MLK3 nucleotide sequence:

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Certificate of Analysis

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