

Certificate of Analysis

MLK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-964, 14-964-K, 14-964M

Parent Lot # D15HP004N

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 6His-tagged, recombinant, human MLK2, amino acids 1-449 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 82% by SDS-PAGE and Coomassie blue staining. MW = 54kDa.

Specific Activity (Parent lot# D15HP004N): 532U/mg, where one unit of MLK2 active 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: 0.65mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

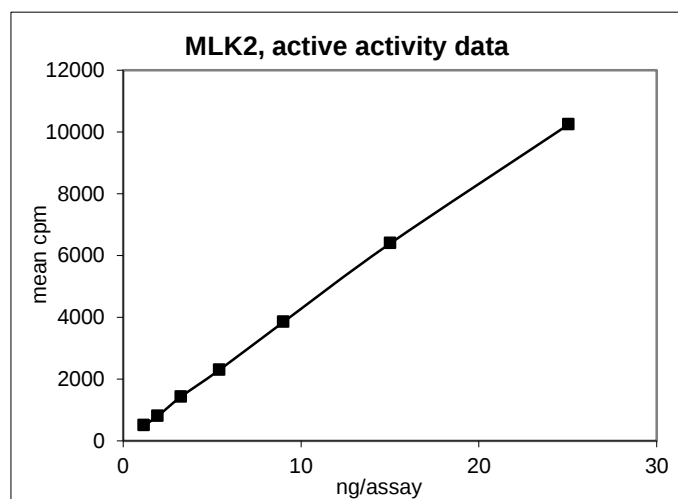
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

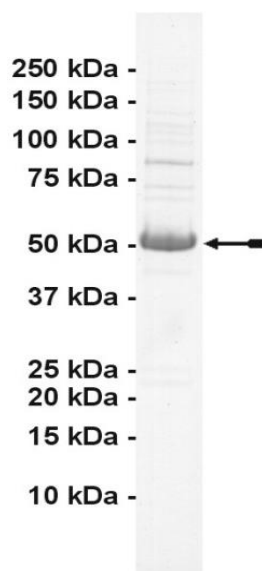
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.2–25ng 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 MLK2 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain:
Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MLK2, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Myelin Basic Protein (MBP):** 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. MLK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.2–25ng per assay point.
- 4. [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 500 - 800cpm/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 MBP.
3. Add 5µl of dH₂O.
4. Add **2.5µl (1.2–25ng) MLK2, active.**
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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.

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MLK2 Sequence Information

Protein	Human MLK2
Tags	N-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of human MLK2
Accession number	GenBank NM_002446.2

Recombinant MLK2 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MEEEGAVAK EWGTTAGPV WTAVFDYEA
61 GDEELTLRRG DRVQVLSQDC AVSGDEGWWT GQLPSGRVGV FPSNYVAPGA PAAPAGLQLP
121 QEIPFHELQL EEIIGVGGFG KVYRALWRGE EVAVKAARLD PEKDPVTA E QVCQEARLFG
181 ALQHPNIIAL RGACLNPPHL CLVMEYARGG ALSRVLAGRR VPPHVLVNW VQVARGMNYL
241 HNDAPVPIIH RDLKSINILI LEAIENHNLA DTVLKITDFG LAREWHKTTK MSAAGTYAWM
301 APEVIRLSLF SKSSDVWSFG VLLWELLTGE VPYREIDALA VAYGVAMNKL TLPIPSTCPE
361 PFARLLEECW DPDPHGRPDF GSILKRLEVI EQSALFQMPL ESFHSLQEDW KLEIQHMFDD
421 LRTKEKELRS REELLRAAQ EQRFAQEQLR RREQELAERE MDIVERELHL LMCQLSQEK

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Recombinant MLK2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggcgcattgga tccggaattc atggaggagg aggagggggc ggtggccaag
121 gagtggggca cgacccccgc gggggccgtc tggaccgcgg tgttcgacta cgaggcggcg
181 ggcgacgagg agctgaccct gcggaggggc gatcgcgctc aggtgctttc ccaagactgt
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301 ttccccagca actacgtggc ccccggcgcc cccgctgcac ccgcgggcct ccagctgccc
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1381 atggacatcg tggaacggga gctgcacctg ctcatgtgcc agctgagcca ggagaagtaa

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