

Certificate of Analysis

MYLK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-966, 14-966-K, 14-966M

Parent Lot # D15JP014N

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: C-terminal 6His-tagged, recombinant, human MYLK2 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 84% by SDS-PAGE and Coomassie blue staining. MW = 66kDa.

Specific Activity (Parent lot# D15JP014N): 3716U/mg, where one unit of MYLK2, active activity is defined as 1nmol phosphate incorporated into 250µM KKLNRTLSFAEPG per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.24mg/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.

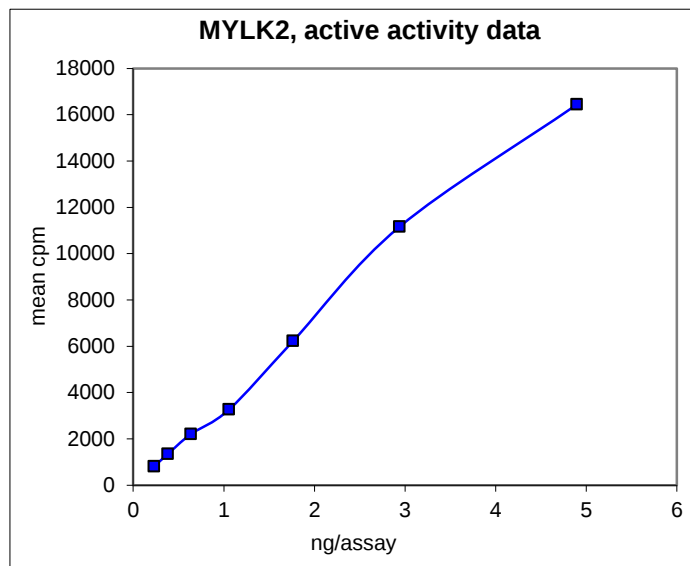
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

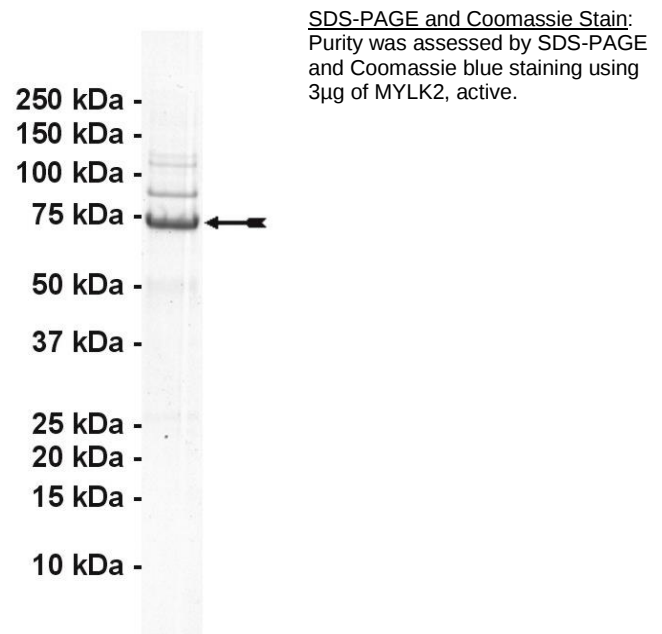
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.2–4.9ng of this lot of enzyme phosphorylated 250µM KKLNRTLSFAEPG 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 MYLK2 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. KKLNRTLSFAEPG:** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
- 3. Calcium Chloride:** Use at a final assay concentration of 0.5mM. Prepare a 50mM stock and use 0.25 μ l per assay point.
- 4. Calmodulin:** Use at a final assay concentration of 0.016mg/ml. Prepare a 0.3mg/ml stock and use 1.325 μ l per assay point.
- 5. MYLK2 active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.2–4.9ng per assay point.
- 6. [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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 3.425 μ l of dH₂O.
3. Add 0.25 μ l of 50mM calcium chloride.
4. Add 1.325 μ l of 0.3mg/ml calmodulin.
5. Add 2.5 μ l of KKLNRTLSFAEPG.
6. Add **2.5 μ l (0.2–4.9ng) MYLK2, active.**
7. Add 10 μ l of diluted [γ -³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
10. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the dried filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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MYLK2, active Sequence Information

Protein	Human MYLK2
Tags	C-terminal 6His
Native sequence	M1 of the recombinant protein is equivalent to M1 of human MYLK2
Accession number	GenBank BC092413.1

Recombinant MYLK2 amino acid sequence:

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1  MATENGAVEL  GIQNPSTDKA  PKGPTGERPL  AAGKDPGPPD  PKKAPDPPTL  KKDAKAPASE
61  KDGDTLAQPS  TSSQGPKGEG  DRGGGPAEGS  AGPPAALPQQ  TATPETS VKK  PKAEQGASGS
121  QDPGKPRVGK  KAAEGQAAAR  RGSPAFLHSP  SCPAIISSE  KLLAKKPPSE  ASELTFEGVP
181  MTHSPTDPRP  AKAEEGKNIL  AESQKEVG EK  TPGQAGQAKM  QGDTSRGIEF  QAVPSEKSEV
241  GQALCLTARE  EDCFQILDDC  PPPAPFP HR  MVELRTGNVS  SEFSMNSKEA  LGGGKFGAVC
301  TCMEKATGLK  LAAKVIKKQT  PKDKEMV LLE  IEVMNQLNHR  NLIQLYAAIE  TPHEIVLFME
361  YIEGGELFER  IVD EYHLTE  VDTMV FVRQI  CDGILFMHKM  RVLHLDLKPE  NILCVNTTGH
421  LVKIIDFGLA  RRYNPNEK LK  VNFGTPEFLS  PEVVNYDQIS  DKTDMWSMGV  ITYMLLSGLS
481  PFLGDDDTET  LNNVLSGNWY  FDEETFEAVS  DEAKDFVSNL  IVKDQRARMN  AAQCLAH PWL
541  NNLAEKAKRC  NRRLKSQILL  KKYLMKRRWK  KNFIAVSAAN  RFKKISSSGA  LMALGVGP GP
601  GHHHHHH

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Recombinant MYLK2 nucleotide sequence:

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1  atggcgacag  aaaatggagc  agttgagctg  ggaattcaga  acccatcaac  agacaaggca
61  cctaaaggctc  ccacaggtga  aagacccttg  gctgcaggga  aagaccctgg  cccccagac
121  ccaaagaaag  ctccgatcc  acccaccctg  aagaaagatg  ccaaagcccc  tgcctcagag
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1561  atcgtcaagg  accagagggc  ccgatgaac  gctgccagct  gtctcgccca  tccctggctc
1621  aacaacctgg  cggagaaagc  caaacgctgt  aaccgacgcc  ttaagtccca  gatcttgctt

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Certificate of Analysis

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1681 aagaaatacc tcatgaagag gcgctggaag aaaaacttca ttgctgtcag cgctgccaac
1741 cgcttcaaga agatcagcag ctcgggggca ctgatggctc tgggggtcgg cccggggcct
1801 ggccatcacc atcaccatca ctaa
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