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Certificate of Analysis

MLCK, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-638, 14-638-K, 14-638M
Parent Lot # 1611522

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 MLCK, amino acids 1425–1771, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 88.9% by SDS-PAGE and Coomassie blue staining. MW = 43.7kDa.

Specific Activity (Parent lot# 1611522): 224U/mg, where one unit of MLCK activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.76mg/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.

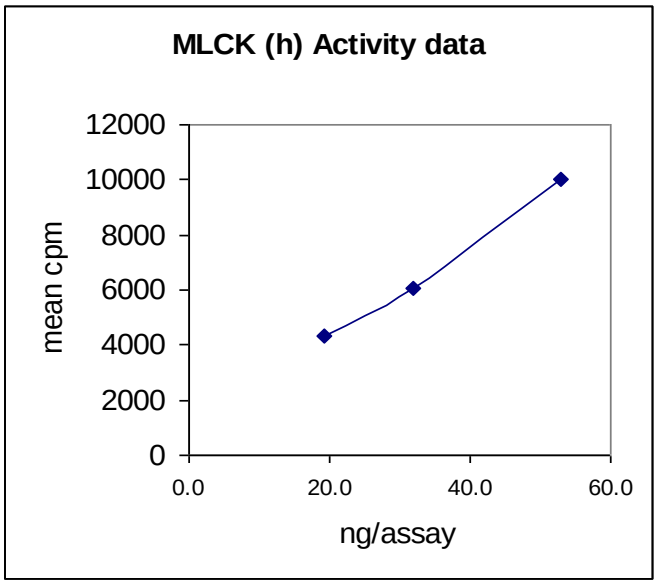
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

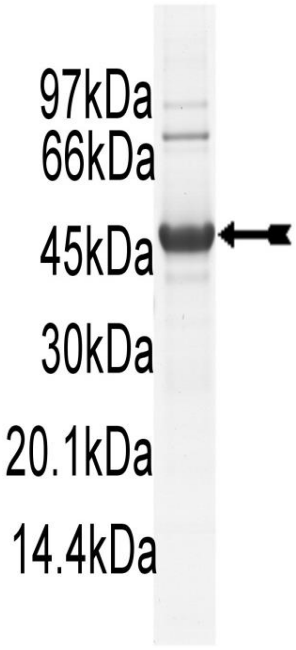
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 19.2–53ng 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 MLCK with the translated native sequence listed on page three.



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

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKLNRTLSFAEPG):** Use at a final concentration of 250µM. Make a 2.5mM stock. Use 2.5µl of stock solution per assay point.
3. **MLCK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 19.2–53ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)
5. **$CaCl_2$:** Use at a final concentration of 0.5mM. Make a 5mM stock. Use 2.5µl of stock solution per assay point.
6. **Calmodulin:** Use at a final assay concentration of 1µM. Make a 0.3mg/ml stock solution. Use 1.33µl of stock per assay point.

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKLNRTLSFAEPG)**.
3. Add **2.5µl (19.2–53ng) MLCK, active**.
4. Add 2.5µl of 5mM $CaCl_2$.
5. Add 1.33µl of 0.3mg/ml calmodulin.
6. Add 1.17µl of dH_2O.
7. Add 10µl of diluted [γ-^{33}P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl 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 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 1µl of 30% phosphoric acid.

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MLCK Sequence Information

Protein Human MLCK

Tags *N*-terminal 6His

Native sequence G31 of the recombinant protein is equivalent to G1425 of human MLCK

Accession number GenBank NM_053025

Recombinant MLCK amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF GEKPEEPKDE VEVSDDDEKE PEVDYRTVTI
  61 NTEQKVSDFY DIEERLGSGK FGQVFRLVEK KTRKVWAGKF FKAYSAKEKE NIRQEISIMN
 121 CLHHPKLVQC VDAFEEKANI VMVLEIVSGG ELFERIIDED FELTERECIK YMRQISEGVE
 181 YIHKQGIVHL DLKPENIMCV NKTGTRIKLI DFGLARRLEN AGSLKVLFGT PEFVAPEVIN
 241 YEPIGYATDM WSIGVICYIL VSGLSPFMGD NDNETLANVT SATWDFDDEA FDEISDDAKD
 301 FISNLLKKDM KNRLDCTQCL QHPWLMKDTK NMEAKKLSKD RMKKYMARRK WQKTGNAVRA
 361 IGRLSSMAMI SGLSGRK
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Recombinant MLCK nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc ggagagaaac ctgaagagcc gaaggatgaa
 121 gtggaggtgt cagatgatga tgagaaggag cccgaggttg attaccggac agtgacaatc
 181 aatactgaac aaaaagtatc tgacttctac gacattgagg agagattagg atctgggaaa
 241 tttggacagg tctttcgact tgtagaaaag aaaactcgaa aagtctgggc agggaagttc
 301 ttcaaggcat attcagcaaa agagaaagag aatatccggc aggagattag catcatgaac
 361 tgcctccacc accctaagct ggtccagtgt gtggatgcct ttgaagaaaa ggccaacatc
 421 gtcatggtcc tggagatcgt gtcaggaggg gagctgtttg agcgcatcat tgacgaggac
 481 tttgagctga cggagcgtga gtgcatcaag tacatgcggc agatctcgga gggagtggag
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 601 aacaagacgg gcaccaggat caagctcatc gactttggtc tggccaggag gctggagaac
 661 gcggggtctc tgaaggtcct ctttggcacc ccagaatttg tggctcctga agtgatcaac
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1021 cggatgaaga agtacatggc aagaaggaaa tggcagaaaa cgggcaatgc tgtgagagcc
1081 attggaagac tgtcctctat ggcaatgatc tcagggctca gtggcaggaa ataa
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Reviewed and approved by site quality representative.