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Certificate of Analysis

DCAMKL1, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-986, 14-986-K, 14-986M
Parent Lot # D18EP016N

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 DCAMKL1 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Purity 52% by SDS-PAGE and Coomassie blue staining. MW = 85kDa.

Specific Activity (Parent lot# D18EP016N): 419U/mg, where one unit of DCAMKL1 activity is defined as 1nmol phosphate incorporated into 250µM KKLNRTLSFAEPG per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.67mg/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.

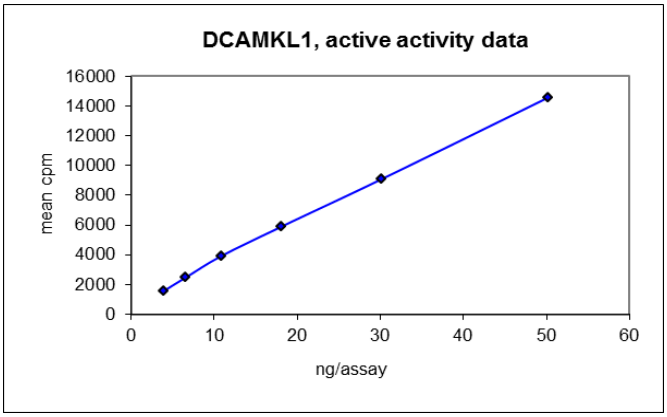
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

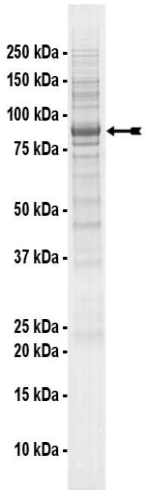
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 3.91–50.25ng 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 DCAMKL1 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 DCAMKL1, 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 assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **DCAMKL1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3.91-50.25ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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).

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 (3.91-50.25ng) DCAMKL1, active.**
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]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 dried 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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DCAMKL1, active Sequence Information

Protein Human DCAMKL1

Tags *N*-terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M1 of human DCAMKL1

Accession number GenBank NM_004734.2

Recombinant DCAMKL1 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MSFGRDMELE HFDERDKAQR YSRGSRVNGL
  61 PSPTHSAHCS FYRTRTLQTL SSEKKAKKVR FYRNGDRYFK GIVYAISPDR FRSFEALLAD
 121 LTRTLSDNVN LPQGVRTIYT IDGLKKISSL DQLVEGESYV CGSIEPFKKL EYTKNVNPNW
 181 SVNVKTTSAS RAVSSLATAK GSPSEVRENK DFIRPKLVTI IRSGVKPRKA VRILLNKKTA
 241 HSFEQVLTDI TDAIKLDSGV VKRLYTLDGK QVMCLQDFFG DDDIFIACGP EKFRYQDDFL
 301 LDESECRVVK STSYTKIASS SRRSTTKSPG PSRRSKSPAS TSSVNGTPGS QLSTPRSGKS
 361 PSPSPTSPGS LRKQRSSQHG GSSTSLASTK VCSSMDENDG PGEEVSEEGF QIPATITERY
 421 KVGRTIGDGN FAVVKECVER STAREYALKI IKKSKCRGKE HMIQNEVSIL RRVKHPNIVL
 481 LIEEMDVPTE LYLVMELVKG GDLFDAITST NKYTERDASG MLYNLASAIK YLHSLNIVHR
 541 DIKPENLLVY EHQDGSKSLK LGDFGLATIV DGPLYTVCGT PTYVAPEIIA ETGYGLKVDI
 601 WAAGVITYIL LCGFPPFRGS GDDQEVLFDQ ILMGQVDFPS PYWDNVSDSA KELITMMLLV
 661 DVDQRFSAVQ VLEHPWVNDD GLPENEHQLS VAGKIKKHFN TGPKPNSTAA GVSVIALDHG
 721 FTIKRSGSLD YYQQPGMYWI RPPLLIRRGR FSDEDATRM
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Recombinant DCAMKL1 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atgtccttcg gcagagacat ggagctggag
 121 cacttcgacg agcgggataa ggcgcagaga tacagccgag ggtcgcgggt gaacggcctg
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 541 tcggtgaacg tcaagaccac ctcggcttct cgggcagtgt cttcactggc cactgccaaa
 601 ggaagccctt cagaggtgcg agagaataag gatttcattc ggcccaagct ggtcaccatc
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 721 cattcctttg agcaggtcct caccgatatc accgatgcca tcaagctgga ctcgggagtg
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1141 ggctcctcta cgtcacttgc gtccaccaaa gtctgcagct cgatggatga gaacgatggc
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1261 aaagtcggaa gaacaatagg agatggaaat tttgctgttg tcaaggaatg tgtagaaaga
1321 tcgactgcta gagagtacgc tctgaaaatt atcaagaaaa gcaaatgtcg aggcaaagag
1381 cacatgatcc agaatgaagt gtctatttta agaagagtga agcatcccaa tatcgttctt
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1441 ctgattgagg agatggatgt gccaactgaa ctgtatcttg tcatggaatt agtaaagggg
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1561 atgctgtaca acctagccag cgccatcaaa tacctgcata gcctgaacat cgtccaccgt
1621 gatatcaagc cagagaacct gctggtgtat gagcaccaag atggcagcaa atcactgaag
1681 ctgggtgact ttggactggc caccattgta gacggccccc tgtacacagt ctgtggcacc
1741 ccaacatacg tggctccaga aatcattgca gagactggat acggcctcaa ggtggacatc
1801 tgggcagcag gtgtaatcac ttatatcctg ctgtgtggtt tccctccatt ccgtggaagt
1861 ggtgatgacc aggaggtgct ttttgatcag attttgatgg ggcaggtgga ctttccttct
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1981 gatgtagatc agcgattttc tgctgttcaa gtacttgagc atccctgggt taatgatgat
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2101 acaggcccca agccgaatag cacagcagct ggagtttctg tcatagcact ggaccacggg
2161 tttaccatca agagatcagg gtctttggac tactaccagc aaccaggaat gtattggata
2221 agaccaccgc tcttgataag gagaggcagg ttttccgacg aagacgcaac caggatgtaa
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