

Certificate of Analysis

DCAMKL3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-943, 14-943-K, 14-943M

Parent Lot # D14CP007N

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 DCAMKL3 amino acids 345-end, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography followed by gel filtration. Purity 97% by SDS-PAGE and Coomassie blue staining. MW = 38kDa.

Specific Activity (Parent lot# D14CP007N): 2527U/mg, where one unit of DCAMKL3, active activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.365mg/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.

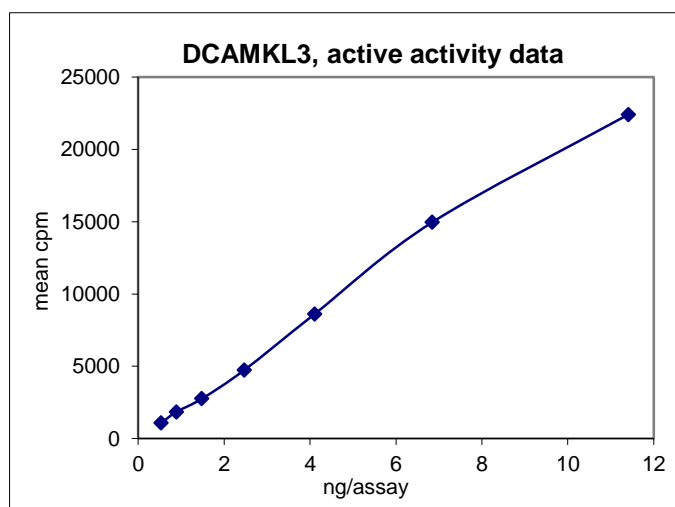
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

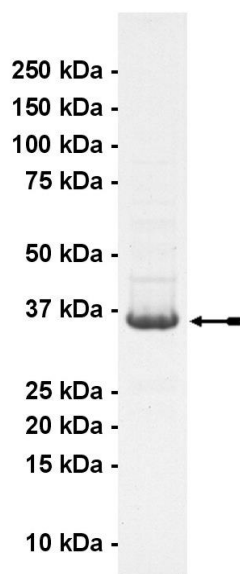
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.5–11.4ng 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 DCAMKL3 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 DCAMKL3, 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 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **DCAMKL3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.5–11.4ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of (KKLNRTLSFAEPG).
3. Add **2.5 μ l (0.5–11.4ng) DCAMKL3, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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DCAMKL3 Sequence Information

| | |
|--------------------------------|---|
| <u>Protein</u> | human DCAMKL3 |
| <u>Tags</u> | N-terminal 6His |
| <u>Native sequence</u> | M31 of the recombinant protein is equivalent to M345 of human DCAMKL3 |
| <u>Accession number</u> | GenBank NM_033403 |

Recombinant DCAMKL3 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MGIIAANVEK HYETGRVIGD GNFAVVKECR
61 HRETRQAYAM KIIDKSRLKG KEDMVDSEIL IIQSLSHPNL VKLHEVYETD MEIYLILEYV
121 QGGDLFDALII ESKVFPEPDA ALMIMDLCKA LVHMHDKSIV HRDLKPENLL VQRNEDKSTT
181 LKLADFLGAK HVRPIFTVC GTPTYVAPEI LSEKGYGLEV DMWAAGVILY ILLCGFPFPR
241 SPERDQDELFI NIIQLGHFEF LPPYWDNISD AAKDLVSRLV VVDPKKRYTA HQVLQHPWIE
301 TAGKTNTVKR QKQVSPSSEG HFRSQHKRVV EQVS

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Recombinant DCAMKL3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc atgggcatca ttgccgcaa tgtggaaaag
121 cattatgaga ctggccgggt cattggggat gggaactttg ctgtcgtgaa ggagtgcaga
181 caccgcgaga ccaggcaggc ctatgcgatg aagatcattg acaagtccag actcaagggc
241 aaggaggaca tgggtggacag tgagatcttg atcatccaga gcctctctca cccaacatc
301 gtgaaattgc atgaagtcta cgaacagac atggaaatct acctgatcct ggagtacgtg
361 caggaggagg acctttttga cgccatcata gaaagtgtga agttcccga gccgatgct
421 gccctcatga tcatggacct atgcaaagcc ctcgtccaca tgcacgacaa gagcattgtc
481 caccgggacc tcaagccgga aaaccttttg gttcagcgaa atgaggacaa atctactacc
541 ttgaaattgg ctgatttttg acttgcaaag catgtggtga gacctatatt tactgtgtgt
601 gggaccccaa cttacgtagc tcccgaatt ctttctgaga aaggttatgg actggagggtg
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841 gtggtagacc ccaaaaagcg ctacacagct catcagggtt ttacgacccc ctggatcgaa
901 acagctggca agaccaatac agtgaaacga cagaagcagg tgtccccag cagcagggtg
961 cacttccgga gccagcaca gagggttgtg gagcaggtat catag

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