

Certificate of Analysis

CaMKK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-022, 15-022-K, 15-022M

Parent Lot # 214877

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 CaMKK1 full length expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 96% by SDS-PAGE and Coomassie blue staining. MW = 60 kDa.

Specific Activity (Parent lot# 214877): 41 U/mg, where one unit of CaMKK1 activity is defined as 1 nmol phosphate incorporated into 0.33 mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100 µM.

Formulation: 3.58 mg/ml of enzyme in 50 mM Tris/HCl pH 7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

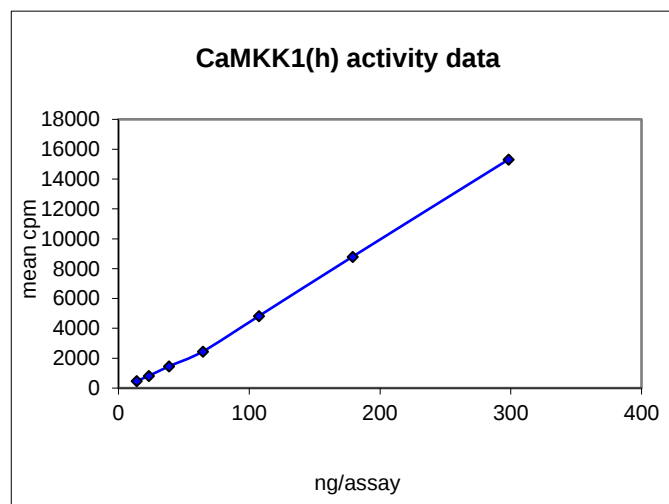
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

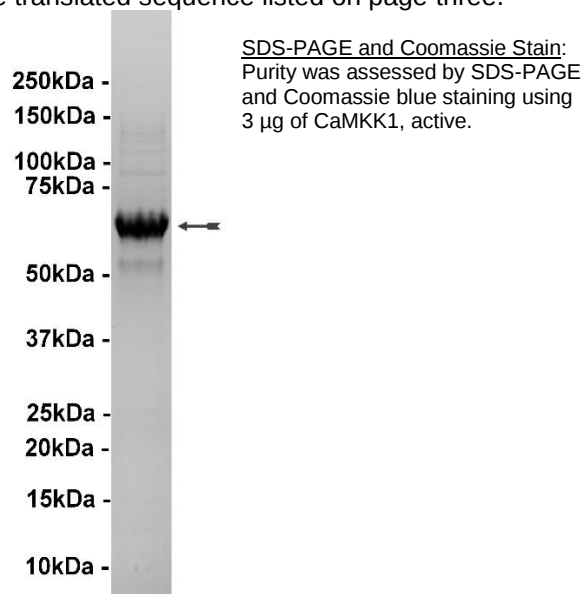
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 13.9–298 ng of this lot of enzyme phosphorylated 0.33 mg/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 CaMKK1 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA.
- 2. Myelin basic protein:** Use at a final assay concentration of 0.33 mg/ml. Prepare a 3.33 mg/ml stock and add 2.5 µl of stock per assay point.
- 3. Calcium chloride:** Use at a final assay concentration of 0.5 mM. Prepare a 50 mM stock and use 0.25 µl per assay point.
- 4. Calmodulin:** Use at a final assay concentration of 0.16 mg/ml. Prepare 0.3 mg/ml stock and use 1.325 µl per assay point.
- 5. CaMKK1, active:** Dilute with 20 mM MOPS/NaOH pH 7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 13.9–298 ng per assay point.
- 6. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 µl of 5 x reaction buffer per assay to wells.
2. Add 2.5 µl of myelin basic protein to wells.
3. Add 0.25 µl of 50 mM calcium chloride to wells.
4. Add 1.325 µl of 0.3 mg/ml calmodulin to wells.
5. Add **2.5 µl (13.9–298 ng) CaMKK1, active.**
6. Add 3.425 µl of dH₂O to wells.
7. Add 10 µl of diluted [γ -³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5 µl of 3% phosphoric acid.
10. Transfer a 10 µl aliquot onto the appropriate area of a **P30 Filtermat.**
11. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the dried filtermat to a sealable plastic bag and add 4 ml 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 µl of 30% phosphoric acid.

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CaMKK1, active Sequence Information

<u>Protein</u>	Human CaMKK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human CaMKK1
<u>Accession number</u>	GenBank NM_172206.1

Recombinant CaMKK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MEGGPAVCCQ DPRAELVERV AAIDVTHLEE
61 ADGGPEPTRN GVDPPPRARA ASVIPGSTSR LLPARPSLSA RKLSLQERPA GSYLEAQAGP
121 YATGPASHIS PRAWRRPTIE SHHVAISDAE DCVQLNQYKL QSEIGKGAYG VVRLAYNESE
181 DRHYAMKVLK KKKLLKQYGF PRRPPPRGSQ AAQGGPAKQL LPLERVYQEI AILKKLDHVN
241 VVKLIEVLDD PAEDNLYLVF DLLRKGPVME VPCDKPFSEE QARLYLRDVI LGLEYLHCQK
301 IVHRDIKPSN LLLGDDGHVK IADFGVSNQF EGNDALQSST AGTPAFMAPE AISDSGQSFS
361 GKALDVWATG VTLYCFVYVK CPFIDDFILA LHRKIKNEPV VFPEEPEISE ELKDLILKML
421 DKNPETRIGV PDIKLHPWVT KNGEEPLPSE EEHCSVVEVT EEEVKNSVRL IPSWTTVILV
481 KSMLRKRSFG NPFEPQARRE ERSMSAPGNL LVKEGFGEKG KSPELPGVQE DEAAS

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Recombinant CaMKK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggcgcattga tccggaattc atggaggggg gtccagctgt ctgctgccag
121 gatcctcggg cagagctggt agaacgggtg gcagccatcg atgtgactca ctggaggagg
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1381 gaggaggagg ttaagaactc agtcaggctc atccccagct ggaccacggt gatcctggtg
1441 aagtccatgc tgaggaagcg ttcttttggg aaccggtttg agcccaagc acggagggaa
1501 gagcgatcca tgtctgctcc aggaaccta ctggtgaaag aagggttttg tgaagggggc
1561 aagagcccag agctccccgg cgtccaggaa gacgaggctg catcctga

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