

Certificate of Analysis

CaMKK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-931, 14-931-K, 14-931M

Parent Lot # D13EP004N

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 CaMKK2 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 85.9% by SDS-PAGE and Coomassie blue staining. MW = 64kDa.

Specific Activity (Parent lot# D13EP004N): 165U/mg, where one unit of CaMKK2, active activity is defined as 1nmol phosphate incorporated into 150µM substrate peptide (LSNLYHQGKFLQTFCGSPLYRRR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.115mg/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.

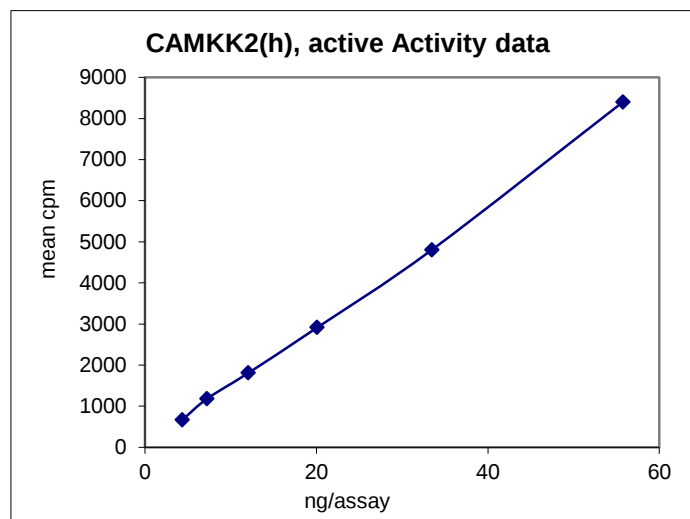
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

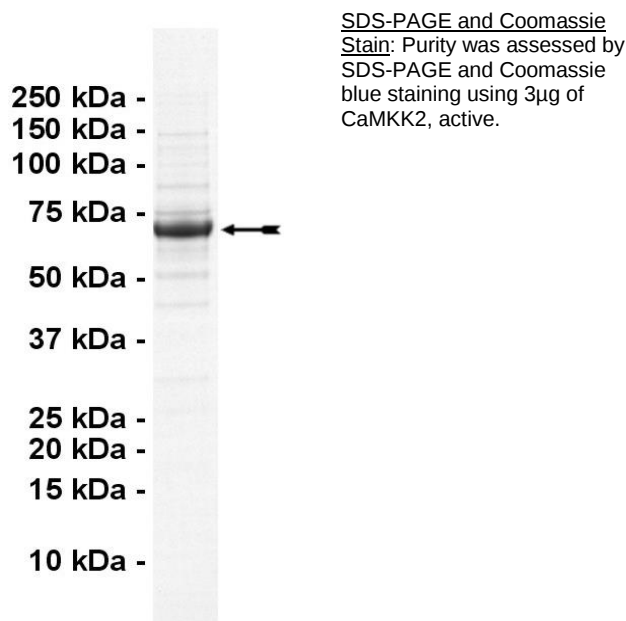
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4–93ng of this lot of enzyme phosphorylated 150µM substrate peptide (LSNLYHQGKFLQTFCGSPLYRRR) 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 CaMKK2 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 100mM Tris/HCl pH8.5, 1mM EDTA
2. **BSA:** Use at a final assay concentration of 0.5%. Make up 10% (100mg/ml) in water and add 1.25µl of stock per assay point
3. **CaCl₂:** Use at a final assay concentration of 0.5mM. Prepare a 50mM stock and add 0.25µl of stock per assay point.
4. **Calmodulin (Catalogue# 14-368):** Use at a final assay concentration of 1µM. Make up a 0.3 mg/ml Stock. Add 1.33µl of stock per assay point.
5. **(LSNLYHQGKFLQTFGSPYRRR):** Use at a final assay concentration of 150µM. Prepare a 1.5mM stock and add 2.5µl of stock per assay point.
6. **CAMKK2, active:** Dilute with 20mM Tris/HCl pH8.5, 0.2mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–93ng per assay point.
7. **[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of (LSNLYHQGKFLQTFGSPYRRR).
3. Add **2.5µl (4–93ng) CaMKK2, active.**
4. Add 1.25µl of 10% BSA
5. Add 0.25µl of CaCl₂.
6. Add 1.33µl of calmodulin.
7. Add 2.17µl of dH₂O.
8. Add 10µl of diluted [γ-³³P]ATP mixture.
9. Incubate for 10 minutes at 30°C.
10. Stop the reaction by adding 5µl of 3% phosphoric acid.
11. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
12. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
13. Wash the filtermat once for 2 minutes with methanol.
14. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
15. 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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CaMKK2 Sequence Information

<u>Protein</u>	human CaMKK2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M37 of the recombinant protein is equivalent to M1 of human CaMKK2
<u>Accession number</u>	GenBank NM_172226 The recombinant protein contains the amino acid substitution T85S with reference to GenBank NM_172226. T85S is reported in GenBank BC026060.

Recombinant CaMKK2 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMSSC VSSQPSSNRA APQDELGGRG
61 SSSSESQKPC EALRGLSSLS IHLGMESFIV VTECEPGCAV DLGLARDRPL EADGQEVPLD
121 SSGSQARPHL SGRKLSLQER SQGGLAAGGS LDMNGRCICP SLPYSPVSSP QSSPRLPRRP
181 TVESHVHSIT GMQDCVQLNQ YTLKDEIGKG SYGVVKLAYN ENDNTYYAMK VLSKKKLIRQ
241 AGFPRRPPPR GTRPAPGGCI QPRGPIEQVY QEIAILKKLD HPNVVKLVEV LDDPNEDHLY
301 MVFELVNQGP VMEVPTLKPL SEDQARFYFQ DLIKGIEYLH YQKIIHRDIK PSNLLVGEDG
361 HIKIADFGVS NEFKGSDALL SNTVGTPAFM APESLSETRK IFSGKALDVW AMGVTLYCFV
421 FGQCPFMDER IMCLHSKIKS QALEFPDQPD IAEDLKDILIT RMLDKNPESR IVVPEIKLHP
481 WVTRHGAEPL PSEDENCTLV EVTEEEVENS VKHIPSLATV ILVKTMIRKR SFGNPFEGSR
541 REERSLSAPG NLLTKQGSSE NLQGTDP PPV GEEEVLL

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Recombinant CaMKK2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgcatgga tccggaattc aaaggcctac gtcgacaaat gtcacatcatgt
121 gtctctagcc agcccagcag caaccggggc gccccccagg atgagctggg gggcaggggc
181 agcagcagca gcgaaagcca gaagccctgt gaggccctgc ggggcctctc atccttgagc
241 atccacctgg gcatggagtc cttcattgtg gtcaccgagt gtgagccggg ctgtgtctgtg
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1261 tttggccagt gccattcat ggacgagcgg atcatgtgtt tacacagtaa gatcaagagt
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1381 cgtatgctgg acaagaaccc cgagtcgagg atcgtgggtc cggaatcaa gctgcacccc
1441 tgggtcacga ggcatggggc ggagccgttg ccgtcggagg atgagaactg cacgctgggtc
1501 gaagtgactg aagaggaggt cgagaactca gtcaaacaca ttcccagctt ggcaaccgtg

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1561 atcctggtga agaccatgat acgtaaacgc tcctttggga acccattcga gggcagccgg
1621 cgggaggaac gtcactgtc agcgcctgga aacttgctca cgaagcaagg cagcgaagac
1681 aacctccagg gcaccgaccc gccccccgtg ggggaggagg aagtgctctt gtga

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