

Certificate of Analysis

ChaK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-961, 14-961-K, 14-961M

Parent Lot # D15DP010N

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 ChaK1 amino acids 1180-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 82kDa.

Specific Activity (Parent lot# D15DP010N): 1895U/mg, where one unit of ChaK1, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml MBP per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.24mg/ml of enzyme in 50mM Tris/HCl pH7.5, 180mM NaCl, 0.09mM EGTA, 270mM sucrose, 259.5mM imidazole, 0.86mM benzamidine, 0.17mM PMSF, 0.09% 2-mercaptoethanol. Frozen solution.

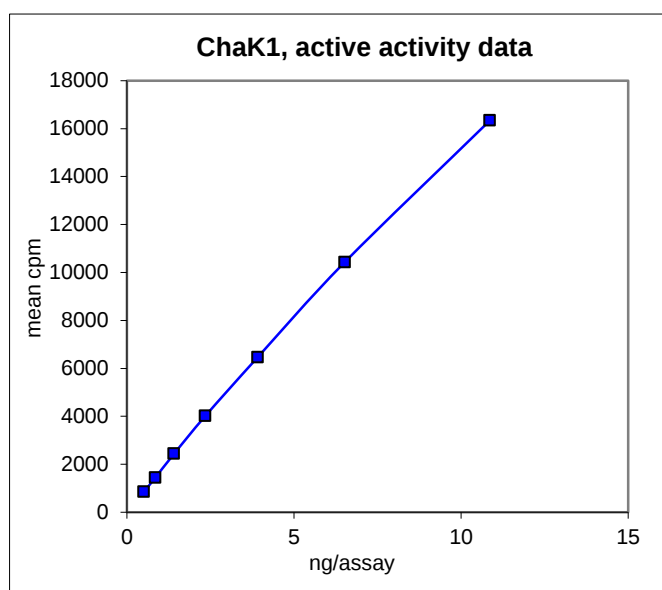
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

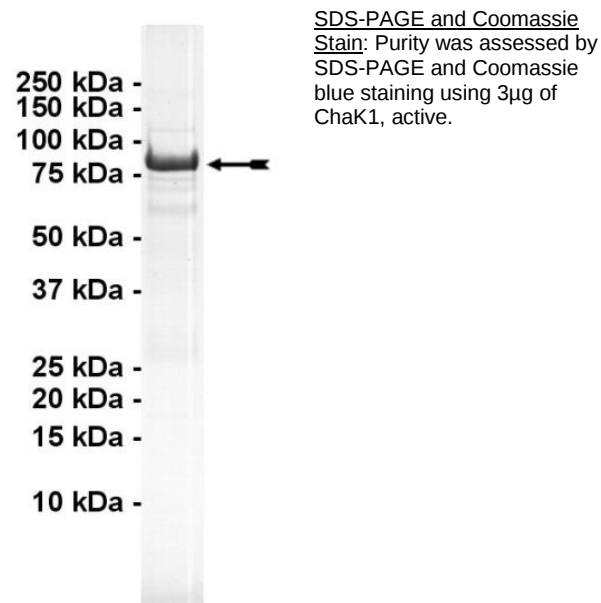
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.5–10.9ng of this lot of enzyme phosphorylated 0.33mg/ml MBP 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 ChaK1 with the translated sequence listed on page three



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Myelin Basic Protein:** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
3. **Manganese Chloride:** Use at a final assay concentration of 5mM. Prepare a 100mM stock and add 1.25µl per assay point.
4. **ChaK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.5–10.9ng per assay point.
5. **[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 3.75µl of dH₂O.
3. Add 2.5µl of MBP.
4. Add 1.25µl of 100mM manganese chloride
5. Add **2.5µl (0.5-10.9ng) ChaK1, active.**
6. Add 10µl of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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ChaK1, active Sequence Information

Protein	human ChaK1
Tags	N-terminal 6His
Native sequence	M37 of the recombinant protein is equivalent to M1180 of human ChaK1.
Accession number	GenBank AY032950.1

Recombinant ChaK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMYFN EKDDKFHSGS EERIRVTFER
61 VEQMCIQIKE VGDRVNYIKR SLQSLDSQIG HLQDLSALTV DTLKTLTAQK ASEASKVHNE
121 ITRELSISKH LAQNLIDDDG VRPSVWKKHG VVNTLSSSLP QGDLESNNPF HCNILMKDDK
181 DPQCNIFGQD LPAVPQRKEF NFPEAGSSSG ALFPSAVSPP ELRQRLHGVE LLKIFNKNQK
241 LGSSSTSIPH LSSPPTKFFV STPSQPSCKS HLETGTDQDE TVCSKATEGD NTEFGAFVGH
301 RDSMDLQRFK ETSNLIKILS NNNTSENTLK RVSSLAGFTD CHRTSIPVHS KQAEKISRPP
361 STEDTHEVDS KAALIPDWLQ DRPSNREMPs EEGTLNGLTS PFKPAMDTNY YYSAVERNNL
421 MRLSQSIPFT PVPPRGEPTV VYRLEESSPN ILNNSMSSWS QLGLCAKIEF LSKEEMGGGL
481 RRAVKVQCTW SEHDILKSGH LYIIKSFLPE VVNTWSSIYK EDTVLHLCLR EIQQQRAAQK
541 LTFAFNQMKP KSIPYSPRFL EVFLLYCHSA GQWFAVEECM TGEFRKYNNN NGDEIIPNT
601 LEEIMLAFSH WTYEYTRGEL LVLDLQGVGE NLTDPSVIKA EEKRSCDMVF GPANLGEDAI
661 KNFRAKHHCN SCCRKLKLPD LKRNDYTPDK IIFPQDEPSD LNLQPGNSTK ESESTNSVRL
721 ML

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Recombinant ChaK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggcgccatgga tccggaattc aaaggcctac gtcgacaaat gtatttcaat
121 gaaaaagatg acaaatttca ttctgggagt gaagagagaa ttcgtgtcac ttttgaaaga
181 gtggaacaga tgtgcattca gattaaagaa gttggagatc gtgtcaacta cataaaaaga
241 tcattacaat cattagattc tcaaatggc catttgcaag atctttcagc cctgacggta
301 gatacattaa aaacactcac tgcccagaaa gcgtcggaag ctagcaaagt tcataatgaa
361 atcacacgag aactgagcat ttccaaacac ttggctcaaa accttattga tgatggctct
421 gtaagacctt ctgtatggaa aaagcatggt gttgtaaata cacttagctc ctctcttctt
481 caaggtgatc ttgaaagtaa taatcctttt cattgtaata ttttaatgaa agatgacaaa
541 gatccccagt gtaatatatt tggtaacagc ttacctgcag taaccagagc aaaagaattt
601 aattttccag aggctggttc ctctcttggt gccttattcc caagtgtgtg ttcccctcca
661 gaactgcgac aggactacata tggggtagaa ctcttaaaaa tatttaataa aaatcaaaaa
721 ttaggcagtt catctactag cataccacat ctgtcatccc caccaaccaa attttttgtt
781 agtacaccat ctacgccaag ttgcaaaagc cacttggaag ctggaaccaa agatcaagaa
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961 aataacaata cttctgaaaa cactttgaaa cgagtgaagt ctcttgctgg atttactgac
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1261 atgagggttat cacagagcat tccatttaca cctgtgcctc caagagggga gcctgtcaca
1321 gtgtatcggt tgggaagagag ttcacccaac atactaaata acagcatgtc ttcttggtca
1381 caactaggcc tctgtgccaa aatagagttt ttaagcaaag aggagatggg aggaggttta
1441 cgaagagctg tcaaagtaca gtgtacctgg tcagaacatg atatcctcaa atcagggcac
1501 ctttatatta tcaaattctt tcttccagag gtggttaata catggtcaag tatttataaa
1561 gaagatacag ttctgcatct ctgtctgaga gaaattcaac aacagagagc agcacaaaag
1621 ctacggtttg cctttaatca aatgaaaccc aaatccatac catattctcc aaggttcctt
1681 gaagttttcc tgctgtattg ccattcagca ggacagtggg ttgctgtgga agaattgatg

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1741 actggagaat ttagaaaata caacaataat aatggagatg agattattcc aactaatact
1801 ctggaagaga tcatgctagc ctttagccac tggacttacg aatatacaag aggggagtta
1861 ctggtacttg atttgcaagg tgttggtgaa aatttgactg acccatctgt gataaaagca
1921 gaagaaaaga gatcctgtga tatggttttt ggcccagcaa atctaggaga agatgcaatt
1981 aaaaacttca gagcaaaaaca tcaactgtaat tcttgctgta gaaagcttaa acttccagat
2041 ctgaagagga atgattatac gcctgataaa attatatttc ctcaggatga gccttcagat
2101 ttgaatcttc agcctggaaa ttccaccaa gaatcagaat caactaattc tggtcgtctg
2161 atgttataa
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