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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.

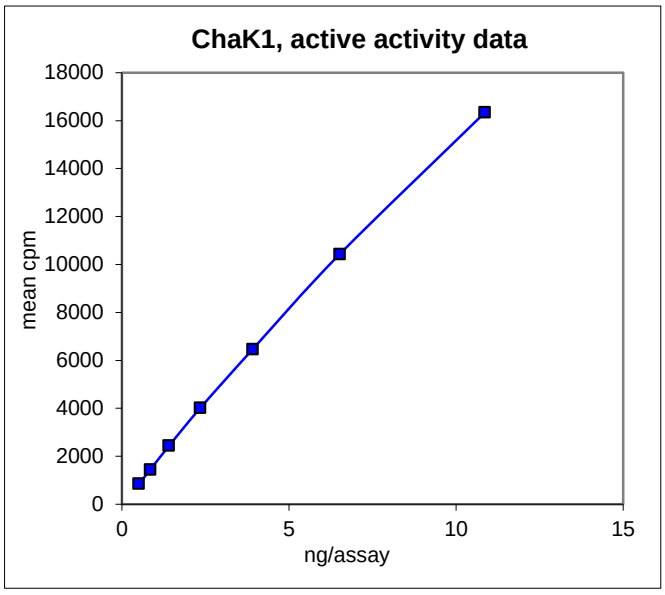
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

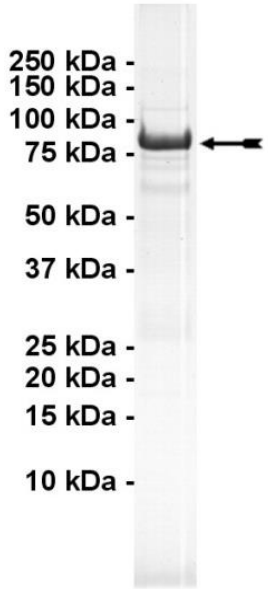
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of ChaK1, active.

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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-^{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 3.75µl of dH_2O.
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-^{33}P]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:

```
   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMYFN EKDDKFHSGS EERIRVTFER
  61 VEQMCIQIKE VGDRVNYIKR SLQSLDSQIG HLQDLSALTV DTLKTLTAQK ASEASKVHNE
 121 ITRELSISKH LAQNLIDDGP VRPSVWKKHG VVNTLSSSLP QGDLESNNPF HCNILMKDDK
 181 DPQCNIFGQD LPAVPQRKEF NFPEAGSSSG ALFPSAVSPP ELRQRLHGVE LLKIFNKNQK
 241 LGSSSTSIPH LSSPPTKFFV STPSQPSCKS HLETGTKDQE TVCSKATEGD NTEFGAFVGH
 301 RDSMDLQRFK ETSNKIKILS NNNTSENTLK RVSSLAGFTD CHRTSIPVHS KQAEKISRRP
 361 STEDTHEVDS KAALIPDWLQ DRPSNREMPS EEGTLNGLTS PFKPAMDTNY YYSAVERNNL
 421 MRLSQSIPFT PVPPRGEPVT VYRLEESSPN ILNNSMSSWS QLGLCAKIEF LSKEEMGGGL
 481 RRAVKVQCTW SEHDILKSGH LYIIKSFLPE VVNTWSSIYK EDTVLHLCLR EIQQQRAAQK
 541 LTFAFNQMKP KSIPYSPRFL EVFLLYCHSA GQWFAVEECM TGEFRKYNNN NGDEIIPTNT
 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 gcgccatgga tccggaattc aaaggcctac gtcgacaaat gtatttcaat
 121 gaaaaagatg acaaatttca ttctgggagt gaagagagaa ttcgtgtcac ttttgaaaga
 181 gtggaacaga tgtgcattca gattaaagaa gttggagatc gtgtcaacta cataaaaaga
 241 tcattacaat cattagattc tcaaattggc catttgcaag atctttcagc cctgacggta
 301 gatacattaa aaacactcac tgcccagaaa gcgtcggaag ctagcaaagt tcataatgaa
 361 atcacacgag aactgagcat ttccaaacac ttggctcaaa accttattga tgatggtcct
 421 gtaagacctt ctgtatggaa aaagcatggt gttgtaaata cacttagctc ctctcttcct
 481 caaggtgatc ttgaaagtaa taatcctttt cattgtaata ttttaatgaa agatgacaaa
 541 gatccccagt gtaatatatt tggtcaagac ttacctgcag taccccagag aaaagaattt
 601 aattttccag aggctggttc ctcttctggt gccttattcc caagtgctgt ttcccctcca
 661 gaactgcgac agagactaca tggggtagaa ctcttaaaaa tatttaataa aaatcaaaaa
 721 ttaggcagtt catctactag cataccacat ctgtcatccc caccaaccaa atttttgtt
 781 agtacaccat ctcagccaag ttgcaaaagc cacttggaaa ctggaaccaa agatcaagaa
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 901 agagatagca tggatttaca gaggtttaaa gaaacatcaa acaagataaa aatactatcc
 961 aataacaata cttctgaaaa cactttgaaa cgagtgagtt ctcttgctgg atttactgac
1021 tgtcacagaa cttccattcc tgttcattca aaacaagcag aaaaaatcag tagaaggcca
1081 tctaccgaag acactcatga agtagattcc aaagcagctt taataccgga ttggttacaa
1141 gatagaccat caaacagaga aatgccatct gaagaaggaa cattaaatgg tctcacttct
1201 ccatttaagc cagctatgga tacaaattac tattattcag ctgtggaaag aaataacttg
1261 atgaggttat cacagagcat tccatttaca cctgtgcctc caagagggga gcctgtcaca
1321 gtgtatcgtt tggaagagag ttcacccaac atactaaata acagcatgtc ttcttggtca
1381 caactaggcc tctgtgccaa aatagagttt ttaagcaaag aggagatggg aggaggttta
1441 cgaagagctg tcaaagtaca gtgtacctgg tcagaacatg atatcctcaa atcagggcat
1501 ctttatatta tcaaatcttt tcttccagag gtggttaata catggtcaag tatttataaa
1561 gaagatacag ttctgcatct ctgtctgaga gaaattcaac aacagagagc agcacaaaag
1621 cttacgtttg cctttaatca aatgaaaccc aaatccatac catattctcc aaggttcctt
1681 gaagttttcc tgctgtattg ccattcagca ggacagtggt ttgctgtgga agaatgtatg
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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 gagcaaaaca tcactgtaat tcttgctgta gaaagcttaa acttccagat
2041 ctgaagagga atgattatac gcctgataaa attatatttc ctcaggatga gccttcagat
2101 ttgaatcttc agcctggaaa ttccaccaaa gaatcagaat caactaattc tgttcgtctg
2161 atgttataa
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Reviewed and approved by site quality representative.