

Certificate of Analysis

CHK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-346, 14-346-K, 14-346M

Parent Lot # 31076U

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 GST tagged, human full length CHK1, expressed in Sf21 insect cells. Purified using glutathione-agarose. Purity 82% by SDS-PAGE and Coomassie blue staining. MW = 81.6kDa.

Specific Activity (Parent lot# 31076U): 687U/mg, where one unit of CHK1 activity is defined as 1nmol phosphate incorporated into 100µM CHKtide (KKKVSRSGLYRSPSPENLNRPR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.877mg/ml of enzyme in 50mM Tris/HCl pH 7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

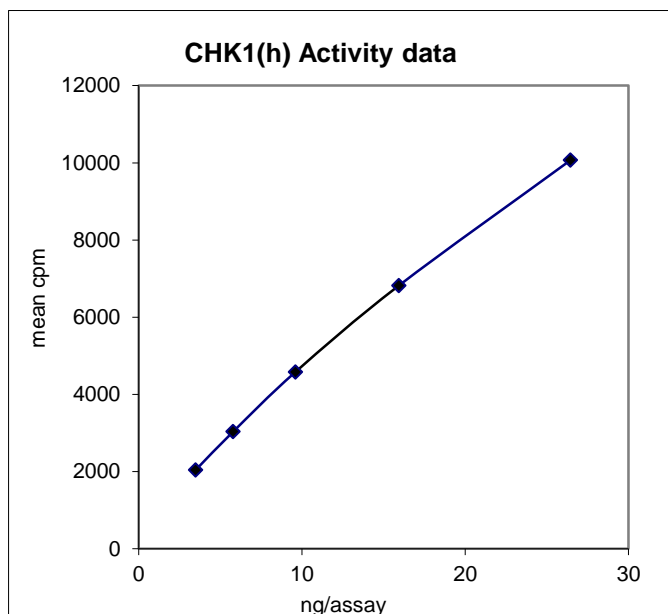
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

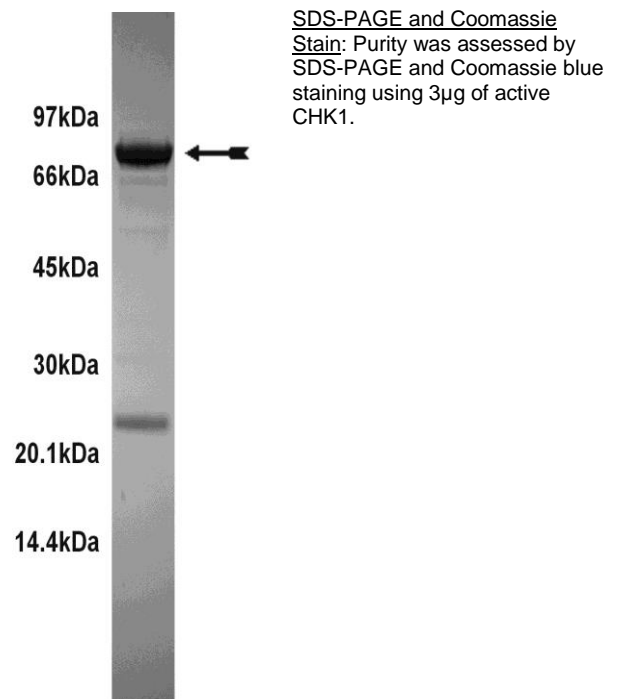
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 11–22ng of this lot of enzyme phosphorylated 100µM CHKtide 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 CHK1 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS pH7.0, 1mM EDTA.
- 2. CHKtide (KKKVSRSGLYRSPSPENLNRPR):** Use at a final assay concentration of 100 μ M. Prepare a 1mM stock. Use 2.5 μ l of stock per assay point.
- 3. CHK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 11–22ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x magnesium acetate/[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 appropriate wells.
2. Add 5 μ l of **CHKtide**.
3. Add **2.5 μ l (11–22ng) CHK1, active**.
4. Add 2.5 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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CHK1 Sequence Information

<u>Protein</u>	Human CHK1
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M234 of the fusion protein is equivalent to M1 of human CHK1
<u>Accession number</u>	GenBank AF016582

Recombinant CHK1 amino acid sequence:

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1  MPILGYWKIK  GLVQPTRLLL  EYLEEKYEEH  LYERDEGDKW  RNKKFELGLE  FPNLPYYIDG
61  DVKLTQSMAI  IRYIADKHM  LGGCPKERAE  ISMLEGAVLD  IRYGVSRIAY  SKDFETLKVD
121  FLSKLPPEMLK  MFEDRLCHKT  YLNGDHVTHP  DFMLYDALDV  VLYMDPMCLD  AFPKLVCFKK
181  RIEAIPQIDK  YLKSSKYIAW  PLQGWQATFG  GGDHPPKSDL  VPRGSRRASV  GSHMAVPFVE
241  DWDLVQTLGE  GAYGEVQLAV  NRVTEEAVAV  KIVDMKRAVD  CPENIKKEIC  INKMLNHENV
301  VKFYGHRREG  NIQYLFLEYC  SGGELFDRIE  PDIGMPEPDA  QRFFHQLMAG  VVYLVHGIGIT
361  HRDIKPENLL  LDERDNLKIS  DFGLATVFRY  NNRERLLNKM  CGTLPYVAPE  LLKRREFHAE
421  PVDVWSCGIV  LTAMLGELP  WDQPSDSCQE  YSDWKEKKTY  LNPWKKIDSA  PLALLHKILV
481  ENPSARITIP  DIKKDRWYNK  PLKKGAKRPR  VTSGGVSESP  SGFSKHIQSN  LDFSPVNSAS
541  SEENVKYSSS  QPEPRTGLSL  WDTSPSYIDK  LVQGISFSQP  TCPDHMLLNS  QLLGTPGSSQ
601  NPWQRLVKRM  TRFFTCLDAD  KSYQCLKETC  EKLGYQWKKS  CMNQVTISTT  DRRNNKLIFK
661  VNLLEMDDKI  LVDFRLSKGD  GLEFKRHFLK  IKGLIDIVS  SQKVWLPAT

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Recombinant CHK1 nucleotide sequence:

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1  atgcctatac  taggttattg  gaaaattaag  ggccttgtgc  aaccactcgc  acttcttttg
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1801 aaccctggc agcggttggt caaaagaatg acacgattct ttaccaaatt ggatgcagac
1861 aaatcttatc aatgcctgaa agagacttgt gagaagttgg gctatcaatg gaagaaaagt
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2101 agccagaagg tttggcttcc tgccacatga
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