

Certificate of Analysis

CHK2, active

(Recombinant enzyme expressed in *E.coli* cells)

Item # 14-347, 14-347-K, 14-347M

Parent Lot # 1832293

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: Recombinant human CHK2, residues 5–end, containing N-terminal GST and C-terminal 6His-tags. Expressed in *E. coli* cells and purified using glutathione-agarose and Ni²⁺/NTA-agarose. Purity 69.3% by SDS-PAGE and Coomassie staining. MW = 89.6kDa.

Specific Activity (Parent lot# 1832293): 1595U/mg, where one unit of CHK2, active activity is defined as 1nmol phosphate incorporated into 200µM CHKtide (KKKVSRSGLYRSPSPENLNRPR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.004mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

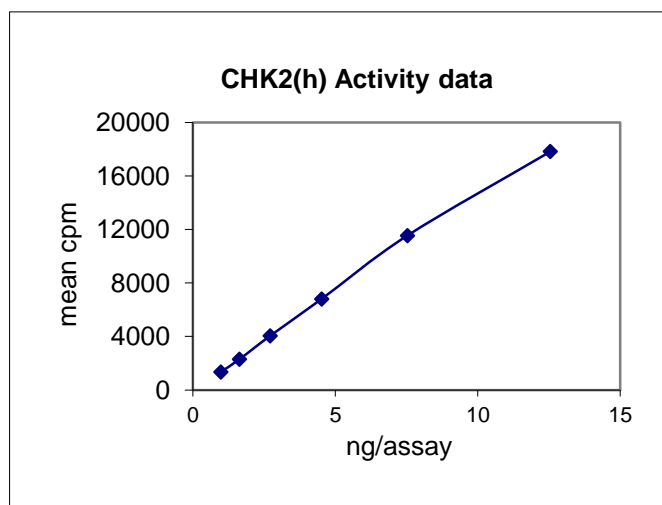
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

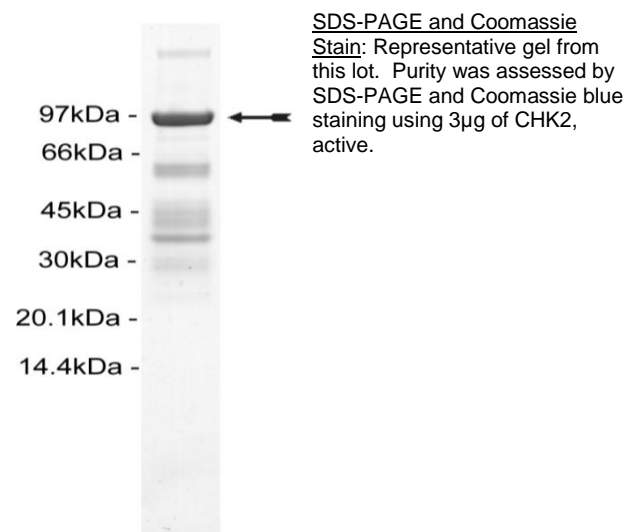
**FOR *IN VITRO* RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1–13ng of this lot of enzyme phosphorylated 200µM CHKtide (KKKVSRSGLYRSPSPENLNRPR) 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 CHK2 with the translated native 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. **CHKtide:** Use at a final assay concentration of 200 μ M. Prepare a 2mM stock and add 2.5 μ l of stock per assay point.
3. **CHK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–13ng 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 well.
2. Add 2.5 μ l of **CHKtide**.
3. Add **2.5 μ l (1–13ng) CHK2, active**.
4. Add 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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CHK2 Sequence Information

<u>Protein</u>	Human CHK2
<u>Tags</u>	N-terminal GST and C-terminal 6His tags
<u>Native sequence</u>	S244 of the fusion protein is equivalent to S5 of human CHK2
<u>Accession number</u>	GenBank NM_007194

Recombinant CHK2 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQ SMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAI PQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSRRAS  VGSHMPMSRP
241 RRP SDVEAQQ  SHGSSACSQP  HGSVTQSQGS  SSQSQGISS  STSTMPNSSQ  SSHSSSGTLS
301 SLETVSTQEL  YSIPEDQEPE  DQEPEEPTPA  PWARLWALQD  GFANLECVND  NYWFGRDKSC
361 EYCFDEPLLK  RTDKYRTYSK  KHFRIFREVG  PKNSYIAYIE  DHSGNGTFVN  TELVGKGKRR
421 PLNNNSEIAL  SLSRNKVFVF  FDLTVDDQSV  YPKALRDEYI  MSKTLGSGAC  GEVKLAFERK
481 TCKKVAIKII  SKRKFAIGSA  READPALNVE  TEIEILKLN  HPCI IKINF  FDAEDYYIVL
541 ELMEGGELFD  KVVGNKRLKE  ATCKLYFYQM  LLAVQYLHEN  GIIHRDLKPE  NVLLSSQEED
601 CLIKITDFGH  SKILGETSLM  RTLCGTPTYL  APEVLVSVGT  AGYNRAVDCW  SLGVILFICL
661 SGYPPFSEHR  TQVSLKDQIT  SGKYNFIPEV  WAEVSEKALD  LVKKLLVDP  KARFTTEEAL
721 RHPWLQDEDM  KRKFQDLLSE  ENESTALPQV  LAQPSTSRKR  PREGAEGAE  TTKRPAVCAA
781 VLHHHHHH

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Recombinant CHK2 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
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1501  agagaggcag  acccagctct  caatgtttaa  acagaaatag  aaattttgaa  aaagctaaat

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1621 gaattgatgg aagggggaga gctgtttgac aaagtgggtgg ggaataaacg cctgaaagaa
1681 gctacctgca agctctatth ttaccagatg ctcttggctg tgcagtacct tcatgaaaac
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2341 gtgttgcac accatcacca tcactga
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