

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, active activity is defined as 1nmol phosphate incorporated into 100µM CHKtide (KKKVSRSGLYRSPSPENLNRP) 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.

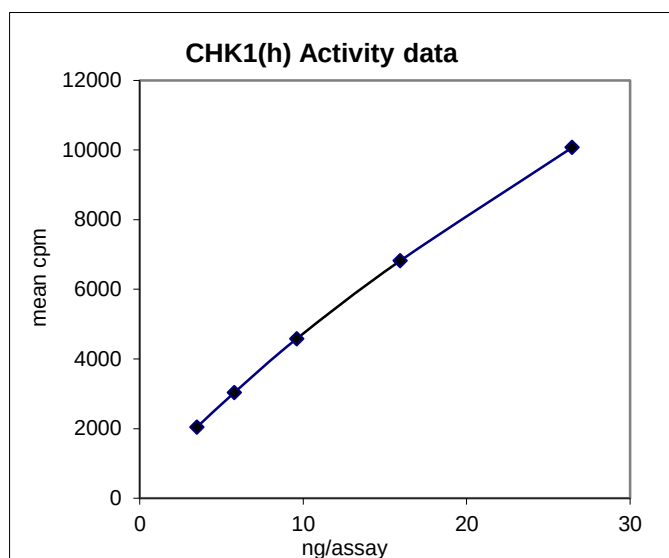
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

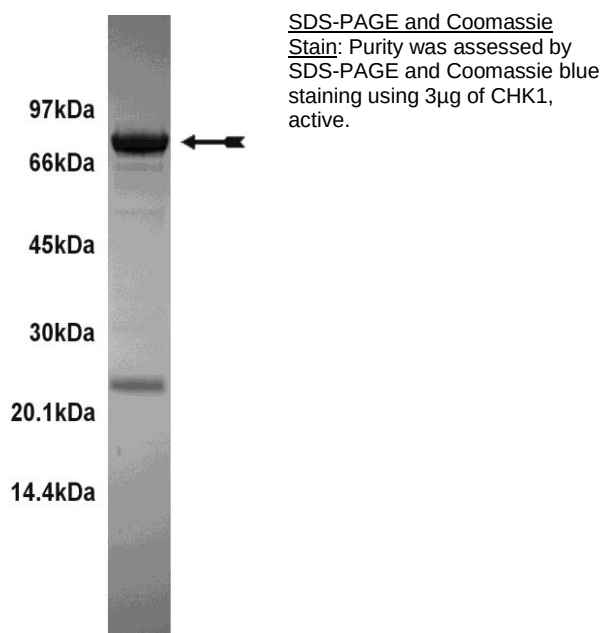
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 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.



Certificate of Analysis

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.

Certificate of Analysis

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:

```

1  MPILGYWKIK  GLVQPTRLLL  EYLEEKYEEH  LYERDEGDKW  RNKKFELGLE  FPNLPYYIDG
61  DVKLTQSMAI  IRYIADKHNM  LGGCPKERA  ISMLEGAVLD  IRYGVSRIAY  SKDFETLKVD
121  FLSKLPPEMLK  MFEDRLCHKT  YLNGDHVTHP  DFMLYDALDV  VLYMDPMCLD  AFPKLVCFKK
181  RIEAIPQIDK  YLKSSKYIAW  PLQGWQATFG  GGDHPPKSDL  VPRGSRRASV  GSHMAVPFVE
241  DWDLVQTLGE  GAYGEVQLAV  NRVTEEAVAV  KIVDMKRAVD  CPENIKKEIC  INKMLNHNEN
301  VKFYGHRREG  NIQYLFLEYC  SGGELFDRIE  PDIGMPEPDA  QRFFHQLMAG  VVYLHGIGIT
361  HRDIKPENLL  LDERDNLKIS  DFGLATVFRY  NNRERLLNKM  CGTLPYVAPE  LLKRREFHAE
421  PVDVWSCGIV  LTAMLAGELP  WDQPSDSCQE  YSDWKEKKTY  LNPWKKIDSA  PLALLHKILV
481  ENPSARITIP  DIKKDRWYNK  PLKKGAKRPR  VTSGGVSESP  SGFSKHIQSN  LDFSPVNSAS
541  SEENVKYSSS  QPEPRTGLSL  WDTSPSYIDK  LVQGIFSFSQP  TCPDHMLLNS  QLLGTPGSSQ
601  NPWQRLVKRM  TRFFTKLDAD  KSYQCLKETC  EKLGYQWKKS  CMNQVTISTT  DRRNNKLIFK
661  VNLEMDDKI  LVDFRLSKGD  GLEFKRHLK  IKGKLIDIVS  SQKVWLPAT

```

Recombinant CHK1 nucleotide sequence:

```

1  atgcctatac  taggttattg  gaaaattaag  ggccttgtgc  aaccactcg  acttcttttg
61  gaatatcttg  aagaaaaata  tgaagagcat  ttgtatgagc  gcgatgaagg  tgataaatgg
121  cgaaacaaaa  agtttgaatt  gggtttgag  tttcccaatc  ttccttatta  tattgatggt
181  gatgttaaat  taacacagtc  tatggccatc  atacgttata  tagctgacaa  gcacaacatg
241  ttgggtggtt  gtccaaaaga  gcgtgcagag  atttcaatgc  ttgaaggagc  ggttttggat
301  attagatacg  gtgtttcgag  aattgcatat  agtaaagact  ttgaaactct  caaagttgat
361  tttcttagca  agctacctga  aatgctgaaa  atgttcgaag  atcgtttatg  tcataaaaca
421  tatttaaatg  gtgatcatgt  aaccatcct  gacttcatgt  tgtatgacgc  tcttgatggt
481  gttttataca  tggaccaaat  gtgcctggat  gcgttcccaa  aattagtttg  ttttaaaaaa
541  cgtattgaag  ctatcccaca  aattgataag  tacttgaaat  ccagcaagta  tatagcatgg
601  cctttgcagg  gctggcaagc  cacgtttggt  ggtggcgacc  atcctccaaa  atcggatctg
661  gttccgcgtg  gatctcgtcg  tgcactgtgt  ggatcgcata  tggcagtgcc  gtttgtggaa
721  gactgggact  tggtgcaaac  cctgggagaa  ggtgcctatg  gagaagttca  acttgcctgtg
781  aatagagtaa  ctgaagaagc  agtcgcagtg  aagattgtag  atatgaagcg  tgccgtagac
841  tgtccagaaa  atattaagaa  agagatctgt  atcaataaaa  tgctaaatca  tgaaaatgta
901  gtaaaattct  atggtcacag  gagagaagcg  aatatccaat  atttatttct  ggagactgtg
961  agtggaggag  agctttttga  cagaatagag  ccagacatag  gcatgcctga  accagatgct
1021  cagagattct  tccatcaact  catggcaggg  gtggtttatc  tgcatggtat  tggaataact
1081  cacagggata  ttaaaccaga  aaatcttctg  ttggatgaaa  gggataacct  caaaatctca
1141  gactttggct  tggcaacagt  atttcggtat  aataatcgtg  agcgtttgtt  gaacaagatg
1201  tgtggtactt  taccatatgt  tgctccagaa  cttctgaaga  gaagagaatt  tcatgcagaa
1261  ccagttgatg  tttggtcctg  tggaatagta  cttactgcaa  tgctcgttgg  agaattgcca
1321  tgggaccaac  ccagtgacag  ctgtcaggag  tattctgact  ggaaagaaaa  aaaaacatac
1381  ctcaaccctt  ggaaaaaaat  cgattctgct  cctctagctc  tgctgcataa  aatcttagtt
1441  gagaatccat  cagcaagaat  taccattcca  gacatcaaaa  aagatagatg  gtacaacaaa
1501  cccctcaaga  aaggggcaaa  aaggccccga  gtcacttcag  gtggtgtgtc  agagtctccc
1561  agtggatttt  ctaagcacat  tcaatccaat  ttggacttct  ctccagtaaa  cagtgtctct
1621  agtgaagaaa  atgtgaagta  ctccagttct  cagccagaac  cccgcacagg  tctttcctta
1681  tgggatacca  gccctcata  cattgataaa  ttggtacaag  ggatcagctt  ttcccagccc
1741  acatgtcctg  atcatatgct  tttgaatagt  cagttacttg  gcacccagg  atcctcacag

```

Certificate of Analysis

```
1801 aacccctggc agcggttggt caaaagaatg acacgattct ttaccaaatt ggatgcagac
1861 aaatcttatc aatgcctgaa agagacttgt gagaagttgg gctatcaatg gaagaaaagt
1921 tgtatgaatc aggttactat atcaacaact gataggagaa acaataaact ctttttcaa
1981 gtgaatttgt tagaaatgga tgataaaata ttggttgact tccggctttc taagggatgat
2041 ggattggagt tcaagagaca cttcctgaag attaaagga agctgattga tattgtgagc
2101 agccagaagg tttggcttcc tgccacatga
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 **Eurofins Pharma Discovery Services UK Limited** is an independent member of Eurofins Discovery Services.