

Certificate of Analysis

CHK2 (R145W), active

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

Item # 14-740, 14-740-K, 14-740M

Parent Lot # D8KN083N

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 and C-terminal 6His-tagged, recombinant human CHK2, residues 5–end, containing the R145W mutation. Expressed in *E. coli* cells. Purified using glutathione-agarose and gel filtration.

This R145W mutation is in the forkhead homology-associated (FHA) domain of CHK2 that has been identified in patients with Li-Fraumeni syndrome (a highly penetrant familial cancer phenotype). The R145W mutant retains some basal kinase activity, however cannot be phosphorylated at an ATM-dependant phosphorylation site and cannot be activated by DNA damage following gamma irradiation.

(Wu X *et al*, JBC (2001);**276**:2971-2974 and Lee SB *et al*, Cancer Research (2001);**61**:8062-8067)

Purity 62% by SDS-PAGE and Coomassie staining. MW = 89.6kDa.

Formulation: 0.262mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM 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.

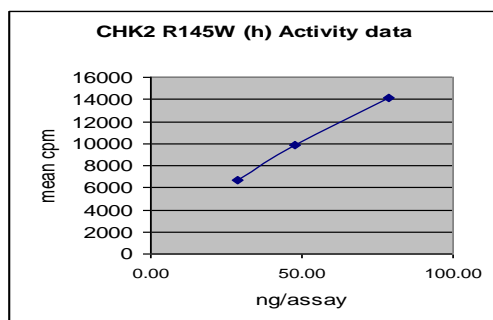
Specific Activity (Parent lot# D8KN083N): 143U/mg, where one unit of CHK2 (R145W), active activity is defined as 1nmol phosphate incorporated into 100µM CHKtide (KKKVSRSGLYRSPSPENLNRPR) per minute at 30°C with a final ATP concentration of 100µM

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.

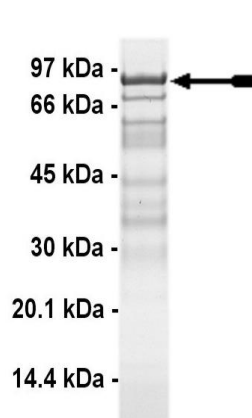
**FOR *IN VITRO* RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 29–79ng of this lot of enzyme phosphorylated 100µM CHKtide (R145W) (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, (R145W) with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of CHK2.(R145W), active

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA
2. **(KKKVSRSGLYRSPSPENLRPR):** Use at a final assay concentration of 100 μ M. Prepare a 1mM stock and add 2.5 μ l of stock per assay point.
3. **CHK2 (R145W), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 29–79ng 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 wells.
2. Add 2.5 μ l of **(KKKVSRSGLYRSPSPENLRPR)**.
3. Add **2.5 μ l (29–79ng) CHK2(R145W), 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 50mM 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 (R145W) Sequence Information

Protein	Human CHK2 (5–end, R145W)
Tags	N-terminal GST and C-terminal 6His tags
Native sequence	S249 of recombinant sequence is equivalent to S5 of native human CHK2
Accession number	GenBank NP_009125

Recombinant CHK2 (R145W) amino acid sequence:

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1 MSPISRMPII  GYWKIKGLVQ  PTRLLLEYLE  EKYEELHYER  DEGDKWRNKK  FELGLEFPNL
61 PYYIDGDVKL  TQSMAIIRYI  ADKHNMLGGC  PKERAEISML  EGAVLDIRYG  VSRIAYSKDF
121 ETLKVDFLSK  LPEMLKMFED  RLCHKTYLNG  DHVTHPDFML  YDALDVVLYM  DPMCLDAFPK
181 LVCFKKRIEA  IPQIDKYLKS  SKYIAWPLQG  WQATFGGGDH  PPKSDLVPRG  SRRASVGSJM
241 PMSRPRRPSD  VEAQQSHGSS  ACSQPHGSVT  QSQSSSSQSQ  GISSSTSTM  PNSSQSSHSS
301 SGTLSLETV  STQELYSIPE  DQEPEDQEPE  EPTPAPWARL  WALQDGFANL  ECVNDNYWFG
361 RDKSCEYCFD  EPLLKRTDKY  RTYSKKHFWI  FREVGPKNSY  IAYIEDHSGN  GTFVNTLVG
421 KGKRRPLNNN  SEIALSLSRN  KVFVFFDLTV  DDQSVYPKAL  RDEYIMSKTL  GSGACGEVKL
481 AFERKTCKKV  AIKIISKRFK  AIGSAREADP  ALNVETEIEI  LKKLNHPCII  KIKNFFDAED
541 YYIVLELMEG  GELFDKVVGN  KRLKEATCKL  YFYQMLLAVQ  YLHENGIIHR  DLKPENVLLS
601 SQEEDCLIKI  TDFGHSKILG  ETSLMRTLGG  TPTYLAPEVL  VSVGTAGYNR  AVDCWSLGI
661 LFICLSGYPP  FSEHRTQVSL  KDQITSGKYN  FIPEVWAEVS  EKALDLVKKL  LVVDPKARFT
721 TEEALRHPWL  QEDMKRKFQ  DLLSEENEST  ALPQVLAQPS  TSRKRPREG  AEGAETTKRP
781 AVCAAVLHHH  HHH

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Recombinant CHK2 (R145W) nucleotide sequence:

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1 atgtccccta  tatctagaat  gcctatacta  ggttattgga  aaattaaggg  ctttgtgcaa
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121 gatgaagggtg  ataaatggcg  aaacaaaaag  tttgaattgg  gtttgagatt  tcccaatctt
181 cttattata  ttgatgggga  tgttaaatta  acacagtcta  tggccatcat  acgttatata
241 gctgacaagc  acaacatggt  ggggtggtgt  ccaaaagagc  gtgcagagat  ttcaatgctt
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361 gaaactctca  aagttgattt  tcttagcaag  ctacctgaaa  tgctgaaaat  gttcgaagat
421 cgtttatgct  ataaaacata  tttaaatggt  gatcatgtaa  cccatcctga  cttcatggtg
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1381 agagatgaat  acatcatgtc  aaaaactctt  ggaagtgggt  cctgtggaga  ggtaaagctg
1441 gctttcgaga  ggaaaacatg  taagaaagta  gccataaaga  tcatcagcaa  aaggaagttt
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1681 aaacgcctga aagaagctac ctgcaagctc tttttttacc agatgctctt ggctgtgcag
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2341 gctgtgtgtg ctgctgtgtt gcatcaccat caccatcact ga
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Reviewed and approved by site quality representative.

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