

Certificate of Analysis

CHK2 (I157T), active

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

Item # 14-741, 14-741-K, 14-741M

Parent Lot # D7DN035U

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 I157T mutation. Expressed in *E. coli* and purified using glutathione-agarose and Ni^{2+} /NTA-agarose.

This I157T 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 I157T mutant retains a similar basal kinase activity compared to the wild type protein. (Wu X *et al*, JBC (2001);276:2971-2974

Purity 85.6% by SDS-PAGE and Coomassie staining. MW = 89.6kDa.

Formulation: 1.654mg/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.

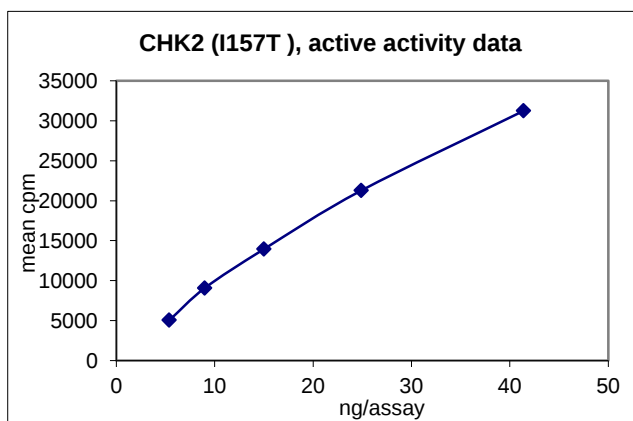
Specific Activity (Parent lot# D7DN035U): 867U/mg, where one unit of CHK2(I157T), active activity is defined as 1nmol phosphate incorporated into 100 μM CHKtide (KKKVSRSGLYRSPSPENLNRP) per minute at 30°C with a final ATP concentration of 100 μM

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 .

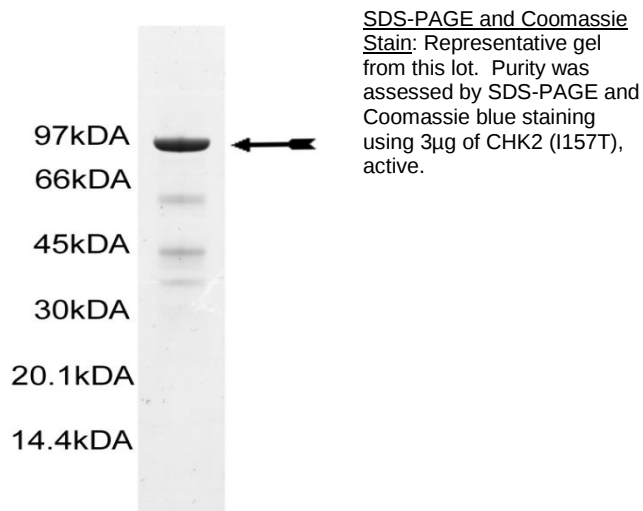
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3–41ng of this lot of enzyme phosphorylated 100 μM CHKtide (KKKVSRSGLYRSPSPENLNRP) 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, (I157T) with the translated native sequence listed on page three.



Certificate of Analysis

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 100 μ M. Prepare a 1mM 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 3–41ng 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 **(KKKVSRSGLYRSPSPENLNRPR)**.
3. Add **2.5 μ l (3–41ng) CHK2 (I157T) 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.

Certificate of Analysis

CHK2 (I157T) Sequence Information

<u>Protein</u>	Human CHK2 (5-end, I157T)
<u>Tags</u>	N-terminal GST and C-terminal 6His tags
<u>Native sequence</u>	S249 of recombinant sequence is equivalent to S5 of native human CHK2
<u>Accession number</u>	GenBank NM 007194

Recombinant CHK2 amino acid sequence:

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1  MSPISRMPII  GYWKIKGLVQ  PTRLLLEYLE  EKYEEHLYER  DEGDKWRNKK  FELGLEFPNL
61  PYYIDGDVKL  TQSMAIIRYI  ADKHNMLGGC  PKERAEISML  EGAVLDIRYG  VSR IAYSKDF
121  ETLKVDFLSK  LPEMLKMFED  RLCHKTYLNG  DHVTHPDFML  YDALDVVLYM  DPMCLDAFPK
181  LVCFKKRIEA  IPQIDKYLKS  SKYIAWPLQG  WQATFGGGDH  PPKSDLVPRG  SRRASVGS HM
241  PMSRPRRPSD  VEAQQSHGSS  ACSQPHGSVT  QSQGSSSQSQ  GISSSTSTM  PNSSQSSHSS
301  SGTLSSETV  STQELYSIPE  DQEPEDQEPE  EPTPAPWARL  WALQDGFANL  ECVNDNYWFG
361  RDKSCEYCFD  EPLLKRTDKY  RTYSKKHFRI  FREVGPKNSY  TAYIEDHSGN  GTFVNTLVG
421  KGKRRPLNNN  SEIALSLSRN  KVFVFFDLTV  DDQSVYPKAL  RDEYIMSKTL  GSGACGEVKL
481  AFERKTCKKV  AIKIISKRF  AIGSAREADP  ALNVETEIEI  LKKLNHPCII  KIKNFFDAED
541  YYIVLELMEG  GELFDKVVG  N KRLKEATCKL  YFYQMLLAVQ  YLHENGIIHR  DLKPENVLLS
601  SQEEDCLIKI  TDFGHSKILG  ETSLMRTL CG  TPTYLAPEVL  VSVGTAGYNR  AVDCWSLGI
661  LFICLSGYPP  FSEHRTQVSL  KDQITSGKYN  FIPEVWAEVS  EKALDLVKKL  LVVDPKARFT
721  TEEALRHPWL  QEDMKRK FQ  DLLSEENEST  ALPQVLAQPS  TSRKRPREGE  AEGAETTKRP
781  AVCAAVLHHH  HHH

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Recombinant CHK2 nucleotide sequence:

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1  atgtccccta  tatctagaat  gcctatacta  gggtattgga  aaattaaggg  ccttgtgcaa
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Certificate of Analysis

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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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