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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 a*l, JBC (2001);**276**:2971-2974
Purity 85.6% by SDS-PAGE and Coomassie staining. MW = 89.6kDa.

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

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.

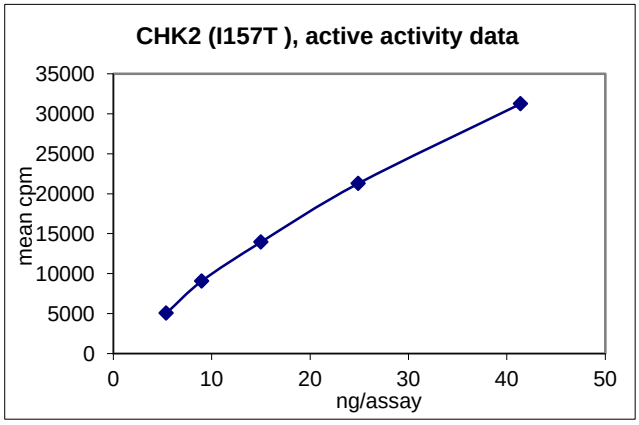
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

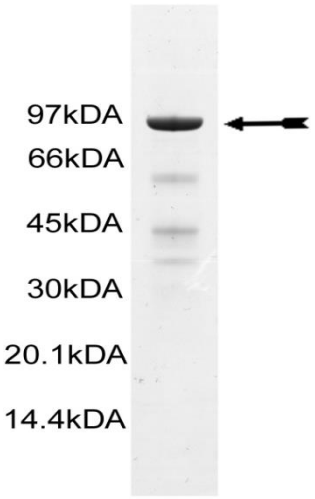
FOR *IN VITRO* RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 3–41ng of this lot of enzyme phosphorylated 100µM CHKtide (KKKVSRSGLYRSPSMPENLNRPR) 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.



SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of CHK2 (I157T), active.

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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 100 µM. Prepare a 1mM stock and add 2.5µl 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-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKKVSRSGLYRSPSMPENLNRPR)**.
3. Add **2.5µl (3–41ng) CHK2 (I157T) active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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CHK2 (I157T) Sequence Information

Protein	Human CHK2 (5-end, I157T)
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 NM 007194

Recombinant CHK2 amino acid sequence:

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  1 MSPISRMPIL GYWKIKGLVQ PTRLLLEYLE EKYEEHLYER DEGDKWRNKK FELGLEFPNL
 61 PYYIDGDVKL TQSMAIIRYI ADKHNMLGGC PKERAEISML EGAVLDIRYG VSRIAYSKDF
121 ETLKVDFLSK LPEMLKMFED RLCHKTYLNG DHVTHPDFML YDALDVVLYM DPMCLDAFPK
181 LVCFKKRIEA IPQIDKYLKS SKYIAWPLQG WQATFGGGDH PPKSDLVPRG SRRASVGSHM
241 PMSRPRRPSD VEAQQSHGSS ACSQPHGSVT QSQGSSSQSQ GISSSSTSTM PNSSQSSHSS
301 SGTLSSLETV STQELYSIPE DQEPEDQEPE EPTPAPWARL WALQDGFANL ECVNDNYWFG
361 RDKSCEYCFD EPLLKRTDKY RTYSKKHFRI FREVGPKNSY TAYIEDHSGN GTFVNTELVG
421 KGKRRPLNNN SEIALSLSRN KVFVFFDLTV DDQSVYPKAL RDEYIMSKTL GSGACGEVKL
481 AFERKTCKKV AIKIISKRKF AIGSAREADP ALNVETEIEI LKKLNHPCII KIKNFFDAED
541 YYIVLELMEG GELFDKVVGN KRLKEATCKL YFYQMLLAVQ YLHENGIIHR DLKPENVLLS
601 SQEEDCLIKI TDFGHSKILG ETSLMRTLCG TPTYLAPEVL VSVGTAGYNR AVDCWSLGVI
661 LFICLSGYPP FSEHRTQVSL KDQITSGKYN FIPEVWAEVS EKALDLVKKL LVVDPKARFT
721 TEEALRHPWL QDEDMKRKFQ DLLSEENEST ALPQVLAQPS TSRKRPREGE AEGAETTKRP
781 AVCAAVLHHH HHH
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Recombinant CHK2 nucleotide sequence:

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   1 atgtccccta tatctagaat gcctatacta ggttattgga aaattaaggg ccttgtgcaa
  61 cccactcgac ttcttttgga atatcttgaa gaaaaatatg aagagcattt gtatgagcgc
 121 gatgaaggtg ataaatggcg aaacaaaaag tttgaattgg gtttggagtt tcccaatctt
 181 ccttattata ttgatggtga tgttaaatta acacagtcta tggccatcat acgttatata
 241 gctgacaagc acaacatgtt gggtggttgt ccaaaagagc gtgcagagat ttcaatgctt
 301 gaaggagcgg ttttggatat tagatacggt gtttcgagaa ttgcatatag taaagacttt
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 421 cgtttatgtc ataaaacata tttaaatggt gatcatgtaa cccatcctga cttcatgttg
 481 tatgacgctc ttgatgttgt tttatacatg gacccaatgt gcctggatgc gttcccaaaa
 541 ttagtttgtt ttaaaaaacg tattgaagct atcccacaaa ttgataagta cttgaaatcc
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 661 cctccaaaat cggatctggt tccgcgtgga tctcgtcgtg catctgttgg atcgcatatg
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1441 gctttcgaga ggaaaacatg taagaaagta gccataaaga tcatcagcaa aaggaagttt
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1501 gctattggtt cagcaagaga ggcagaccca gctctcaatg ttgaaacaga aatagaaatt
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1681 aaacgcctga aagaagctac ctgcaagctc tatttttacc agatgctctt ggctgtgcag
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Reviewed and approved by site quality representative.