KinaseDetect ApS
http://www.kinasedetect.com

Technical support:
Phone: +45 66 10 66 62
e-mail:support@kinasedetect.com

Certificate of Analysis

Product	**CHK2 (CHEK2), active** **human recombinant, expressed in E.coli, N-His-fusion protein**
Cat No	PK-005-01
Lot No	231105
Description	Purified recombinant human checkpoint homologue kinase (CHK2) expressed in E.coli. Active form of CHK2. Suitable for labeling CHK substrates. Features a polyhistidine tag to facilitate removal of CHK2 from the reaction mixture. Purified by Ni-NTA agarose chromatography and gel filtration. Sequence based calculated M.W. 63,964 Approved HUGO gene symbol: CHEK2 Synonyms: CHK2 checkpoint homolog (S. pombe), CDS1, HuCDS1, PP1425, RAD53, bA44G7
Quality	Protein concentration (Bradford with BSA as standard): 0.29 mg/ml Purity: > 90 % by SDS PAGE Specific activity: 40,430 Units*/ mg (* 1 Unit is defined as 1 picomole phosphate transferred to synthetic peptide (KKKVSRSGLYRSPSMPENLNRPR) per min at 30 °C) Protease activity (Twining test): none
Form	Liquid. In 50 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, 0.1 mM EGTA, Protease Inhibitor Cocktail Complete (Roche Cat. Nr. 1 697 498, 5 tablets per liter), 270 mM sucrose, pH 7.5.
Package size	20 microgram
Storage condition	-70 ^{O}C
Shipment conditions	dry ice

Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

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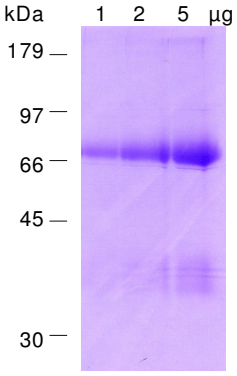
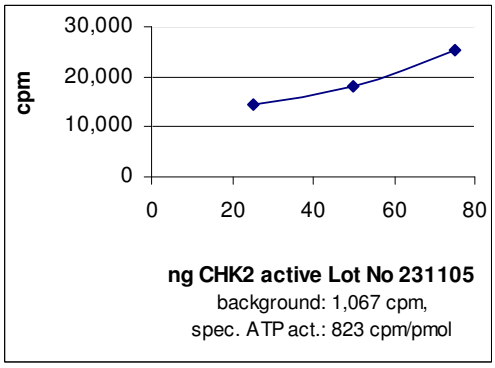
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M31 corresponds to M1 in NM_007194, factor Xa clavage site is underlined

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MRGSHHHHHH GSGSGSGIEG RPYNGTGSAC MSRESDVEAQ QSHGSSACSQ  50
PHGSVTQSQG SSSQSQGISS SSTSTMPNSS QSSHSSSGTL SSLETVSTQE 100
LYSIPEDQEP EDQEPEEPTP APWARLWALQ DGFANLECVN DNYWFGRDKS 150
CEYCFDEPLL KRTDKYRTYS KKHFRIFREV GPKNSYIAYI EDHSGNGTFV 200
NTELVGKGKR RPLNNNSEIA LSLSRNKVFV FFDLTVDDQS VYPKALRDEY 250
IMSKTLGSGA CGEVKLAFER KTCKKVAIKI ISKRKFAIGS AREADPALNV 300
ETEIEILKKL NHPCIIKIKN FFDAEDYYIV LELMEGGELF DKVVGNKRLK 350
EATCKLYFYQ MLLAVQYLHE NGIIHRDLKP ENVLLSSQEE DCLIKITDFG 400
HSKILGETSL MRTLCGTPTY LAPEVLVSVG TAGYNRAVDC WSLGVILFIC 450
LSGYPPFSEH RTQVSLKDQI TSGKYNFIPE VWAEVSEKAL DLVKKLLVVD 500
PKARFTTEEA LRHPWLQDED MKRKFQDLLS EENESTALPQ VLAQPSTSRK 550
RPREGEAEGA ETTKRPAVCA AVL                               573
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In vitro Kinase Assay

Assay components
Reaction buffer: 40 mM MOPS pH 7.5, 2 mM EDTA
Substrate: CHK peptide (KKKVSRSGLYRSPSMPENLNRPR) , 200 microM
Protein kinase: CHK2, 50 - 100 ng/ 3 microliter diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 5 % glycerol, 10 mM DTT, 1 mg/ml BSA
Magnesium/ATP Cocktail: 37.5 mM $MgCl_2$, 250 microM ATP
Diluted [gamma–^{32}P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 microCi) [gamma–^{32}P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay procedure
All compounds are pipetted into a microcentrifuge tube on ice
1. Add 4 microliter reaction buffer
2. Add 3 microliter 200 microM CHK peptide
3. Add 3 microliter CHK2 enzyme (50 - 100 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 30 ^{o}C.
6. Stop the reaction by setting samples on ice
7. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec)
8. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
9. Wash 3 x with phosphoric acid
10. Wash 1 x with acetone
11. Dry under infrared light
12. Read in scintillation counter or Instant Imager.

Reference

Bartek J, Falck J, Lukas J. (2001) Nat Rev Mol Cell Biol. 12: 877-86.