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 Sf9 cells, N-His-fusion protein**
Cat No	PK-006-01
Lot No	080306
Description	Purified recombinant human checkpoint homologue kinase (CHK2) expressed in Sf9 cells. 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. Sequence based calculated M.W. 65,430 Approved HUGO gene symbol: CHEK2 Synonyms: CHK2 checkpoint homolog (S. pombe), CDS1, HuCDS1, PP1425, RAD53, bA44G7
Quality	Protein concentration (Bradford with BSA as standard): 0.4 mg/ml Purity: > 95% by SDS PAGE Specific activity: 142,600 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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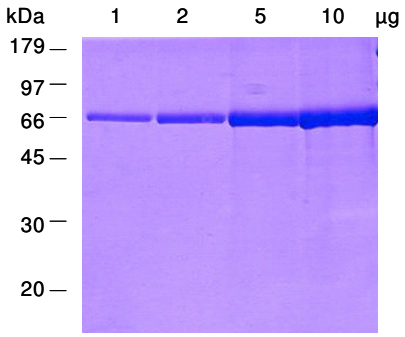
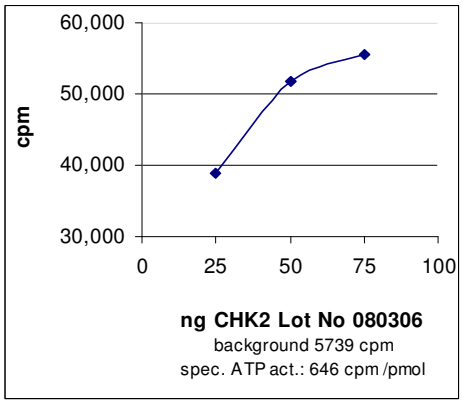
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M31 corresponds to M1 in NM_007194, L573 corresponds to the last amino acid of NM_007194

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MSYYHHHHHHD YDIPTTENLY FQGAMGIRNS MSRESDVEAQ QSHGSSACS  50
QPHGSVTQSQG SSSQSQGISS SSTSTMPNSS QSSHSSSGTL SSLETVSTQ 100
ELYSIPEDQEP EDQEPEEPTP APWARLWALQ DGFANLECVN DNYWFGRDK 150
SCEYCFDEPLL KRTDKYRTYS KKHFRIFREV GPKNSYIAYI EDHSGNGTF 200
VNTELVGKGKR RPLNNNSEIA LSLSRNKVFV FFDLTVDDQS VYPKALRDE 250
YIMSKTLGSGA CGEVKLAFER KTCKKVAIKI ISKRKFAIGS AREADPALN 300
VETEIEILKKL NHPCIIKIKN FFDAEDYYIV LELMEGGELF DKVVGNKRL 350
KEATCKLYFYQ MLLAVQYLHE NGIIHRDLKP ENVLLSSQEE DCLIKITDF 400
GHSKILGETSL MRTLCGTPTY LAPEVLVSVG TAGYNRAVDC WSLGVILFI 450
CLSGYPPFSEH RTQVSLKDQI TSGKYNFIPE VWAEVSEKAL DLVKKLLVV 500
DPKARFTTEEA LRHPWLQDED MKRKFQDLLS EENESTALPQ VLAQPSTSR 550
KRPREGEAEGA ETTKRPAVCA AVLKLVEKY                      580
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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.