

CHK1

CHK1 Checkpoint homolog (*S. pombe*)

Recombinant Human Active Protein Kinase

HGNC Symbol: CHEK1

Synonyms: n/a

Product No.: 0282-0000-1

Lot: 002

Description: Human CHK1, full length, amino acids M₁-T₄₇₆ (as in NCBI/Protein entry NP_001265.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CHK1 Lot 002, was confirmed as CHK1 by specific Western Blotting using anti CHK1 antibody

Theoretical MW_{Fusion Protein}: 83,930 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM TRIS pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C

For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.109 µg/µl

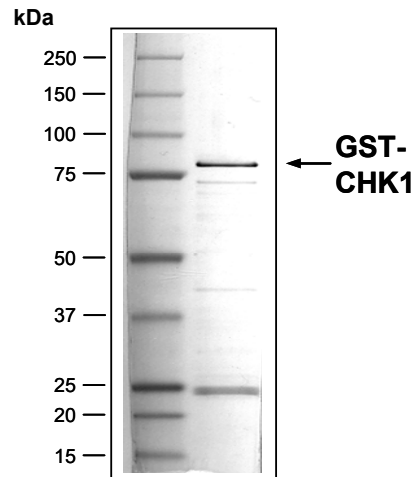
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 20 pmol/µg×min

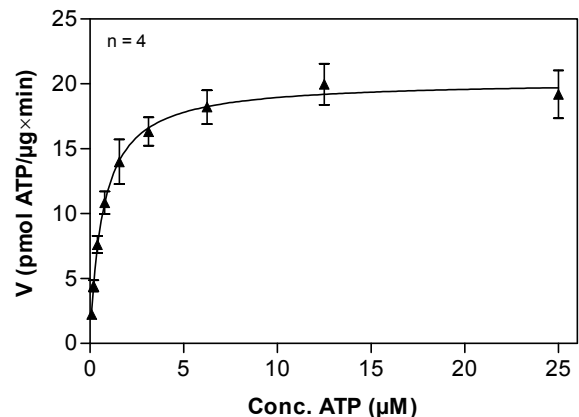
ATP-K_M: 0.69 µM

**CHK1 Lot 002:
Coomassie stain**



1.0 µg GST-CHK1

**CHK1 Lot 002:
Determination of V_{max} and K_M value for ATP**



Determination of K_M value & Specific activity:

- Assay conditions:
 - 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: Substrate: S6-peptide (R₁₁-IAKRRRLSSLRASTSKSESSQK), 40 µg/ml
 - CHK1: 2.0 µg/ml
- Filter binding assay
 - MSPH membrane (Millipore)

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CHK1 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH HG	RRRASVAAGI	240
241	LVPRGS PGLD	GIYAR MAVP	FVEDWDLVQT	LGEGAYGEVQ	LAVNRVTEEA	VAVKIVDMKR	300
301	AVDCPENIKK	EICINKMLNH	ENVVKFYGHR	REGNIQYLFL	EYCSGGELFD	RIEPDIGMPE	360
361	PDAQRFFHQL	MAGVVYLHGI	GITHRDIKPE	NLLDERDNL	KISDFGLATV	FRYNNRERLL	420
421	NKMCGLTPYV	APELLKRREF	HAEPVDVWSC	GIVLTAMLAG	ELPWDQPSDS	CQEYSDWKEK	480
481	KTYLNPWKKI	DSAPLALLHK	ILVENPSARI	TIPDIKKDRW	YNKPLKKGAK	RPRVTSGGVS	540
541	ESPSGFSKHI	QSNLDFSPVN	SASSEENVKY	SSSQPEPTG	LSLWDTSPSY	IDKLVOGISF	600
601	SQPTCPDHML	LNSQLLGTGP	SSQNPWQRLV	KRMTRFFTKL	DADKSYQCLK	ETCEKLGQW	660
661	KKSCMNQVTI	STTDRRNNKL	IFKVNLEMD	DKILVDFRLS	KDGLFVKRH	FLKIKGKLID	720
721	IVSSQKVWLP	AT					780

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:** CHK1

CHK1 wt ¹ Amino Acid Sequence							
1	MAVPFVEDWD	LVQTLGEGAY	GEVQLAVNRV	TEEAVAVKIV	DMKRAVDCPE	NIKKEICINK	60
61	MLNHENVVKF	YGHRREGNIQ	YLFLEYCSGG	ELFDRIEPI	GMPEPDAQRF	FHQLMAGVVY	120
121	LHGIGITHRD	IKPENLLLDE	RDNLKISDFG	LATVFRYNNR	ERLLNKMCGT	LPYVAPELLK	180
181	REFHAEPVD	VWSCGIVLTA	MLAGELPWDQ	PSDSCQEYSD	WKEKTYLNP	WKKIDSAPLA	240
241	LLHKILVENP	SARITIPDIK	KDRWYNKPLK	KGAKRPRVTS	GGVSESPSGF	SKHIQSNLDF	300
301	SPVNSASSE	NVKYSSSQPE	PRTGLSLWDT	SPSYIDKLVO	GISFSQPTCP	DHMLLNSQLL	360
361	GTPGSSQNPW	QLRVKRMTRF	FTKLDADKSY	QCLKETCEKL	GYQWKKSCMN	QVTISTTDRR	420
421	NNKLIKVN	LEMDDKILVD	FRLSKDGL	FKRHFLKIKG	KLIDIVSSQK	VWLPAT	480

blue: CHK1 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_001265.1