

Certificate of Analysis



HCK

hemopoietic cell kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: HCK

Synonyms: JTK9

Product No.: 0408-0000-1

Lot: 001

Description: Human HCK, full length, amino acids M₁-P₅₀₅ (as in NCBI/Protein entry NP_002101.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: HCK Lot 001, was confirmed as HCK by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 86,707 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 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: 0.143 µg/µl

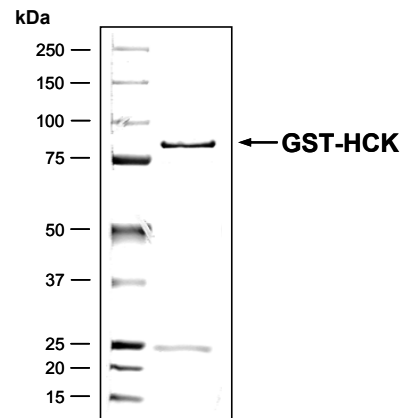
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 34 pmol/µg×min

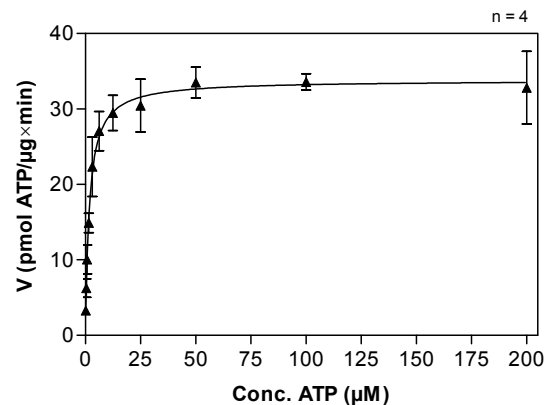
ATP-K_M: 1.8 µM

**HCK Lot 001:
Coomassie stain**



2.0 µg GST-HCK

**HCK Lot 001:
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: Poly(Glu:Tyr)_{4:1} 20 µg/ml
 - HCK: 1.0 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: HCK Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details



HCK

Product No.: 0408-0000-0

HCK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	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	GICSR MGCMK	SKFLQVGGNT	FSKTETSASP	HCPVYVPDPT	STIKPGPNSH	300
301	NSNTPGIREA	GSEDIIVVAL	CDYEAIHHED	LSFQKGDQMV	VLEESGEWWK	ARSLATRKEG	360
361	YIPSNYVARV	DSLETEEFFF	KGISRKDAER	QLLAPGNMLG	SFMIRDSETT	KGSYSLSVRD	420
421	YDPRQGDTVK	HYKIRTLDNG	GFYISPRSTF	STLQELVDHY	KENDGLCQK	LSVPCMSKPK	480
481	QKPWEKDAWE	IPRESLKLEK	KLGAQGFGEV	WMATYNKHTK	VAVKTMKPGS	MSVEAFLAEA	540
541	NVMKTLQHDK	LVKLHAVVTK	EPIYIITEFM	AKGSLDLDFL	SDEGSKQPLP	KLIDFSAQIA	600
601	EGMAFIEQRN	YIHRDLRAAN	ILVSASLVCK	IADFGLARVI	EDNEYTAREG	AKFPIKWTAP	660
661	EAINFGSFTI	KSDVWSFGIL	LMEIVTYGRI	PYPGMSNPEV	IRALERGYRM	PRPENCPEEL	720
721	YNIMMRCWKN	RPEERPTFEY	IQSVLDDFYT	ATESQYQQQP			780

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: HCK **boxed**: variation from RefSeq

HCK wt ¹ amino acid sequence							
1	MGCMKSKFLQ	VGGNTFSKTE	TSASPHCPVY	VPDPTSTIKP	GPNSHNSNTP	GIREAGSEDI	60
61	IVVALYDYEA	IHHEDLSFQK	GDQMVVLEES	GEWWKARSLA	TRKEGYIPSN	YVARVDSLET	120
121	EEWFFKGISR	KDAERQLLAP	GNMLGSFMIR	DSETTKGSYS	LSVRDYDPRQ	GDTVKHYKIR	180
181	TLDNGGFYIS	PRSTFSTLQE	LVDHYKKGND	GLCQKLSVPC	MSSKPKQPWE	KDAWEIPRES	240
241	LKLEKKLGA	QFGEVWMATY	NKHTKVAVKT	MKPGSMSVEA	FLAEANVMKT	LQHDKLVKLH	300
301	AVVTKEPIYI	ITEFMAKGS	LDLFLSDEGS	KQPLPKLIDF	SAQIAEGMAF	IEQRNYIHRD	360
361	LRAANILVSA	SLVCKIADFG	LARVIEDNEY	TAREGAKFPI	KWTAPEAINF	GSFTIKSDVW	420
421	SFGILLMEIV	TYGRIPYPM	SNPEVIRALE	RGYRMPRPEN	CPEELYNIMM	RCWKNRPEER	480
481	PTFEYIQSVL	DDFYTATESQ	YQQQP				540

blue: HCK sequence expressed in fusionprotein **Red**: variant in fusionprotein

¹NCBI/Protein accession number NP_002101.1

G208E: SNP variation see NCBI/dbSNP ID: rs76669962

comment: more recent accession number NP_002101.2 features another initiation codon and a 21aa longer N-terminus