

Certificate of Analysis



ACK1

tyrosine kinase, non-receptor, 2

Recombinant Human Active Protein Kinase

HGNC Symbol: TNK2

Synonyms: p21cdc42Hs

Product No.: 0493-0000-1

Lot: 003

Description: Human ACK1, internal fragment, amino acids G₁₁₀-W₄₇₆ (as in NCBI/Protein entry NP_005772.3), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: ACK1 Lot 003, was confirmed as ACK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 71,130 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM HEPES pH 7.5, 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: 0.165 µg/µl

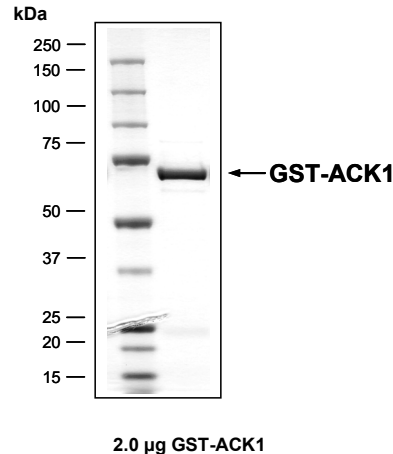
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

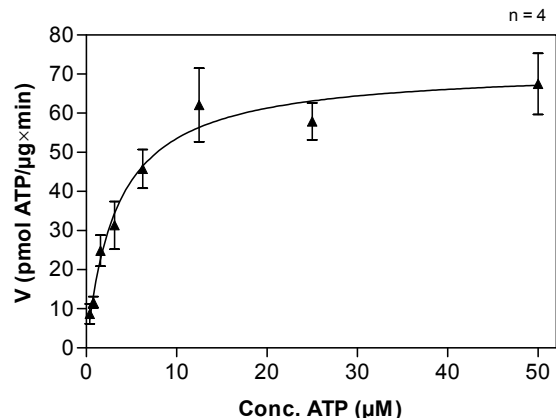
Specific kinase activity (P_i transfer): 72 pmol/µg×min

ATP-K_M: 3.4 µM

**ACK1 Lot 003:
Coomassie stain**



**ACK1 Lot 003:
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} 40 µg/ml
 - ACK1: 4.0 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: ACK1 Lot 003

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from



ACK1

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GST-ACK1 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQG WQATF	GGGDHPPKSD	PMG HHHHHH HG	RRRASVAAGI	240
241	LVPRGS PGLD	GICSR GEGPL	QSLTCLIGEK	DLRLLEKLGD	GSFGVVRGE	WDAPSGKTVS	300
301	VAVKCLKPDV	LSQPEAMDDF	IREVNAMHSL	DHRNLIRLYG	VVLTPPMKMV	TELAPLGSLL	360
361	DRLRKHQGHF	LLGTL SRYAV	QVAEGMGYLE	SKRFIHRDLA	ARNLLLATRD	LVKIGDFGLM	420
421	RALPQNDDHY	VMQEHKVPF	AWCAPESLKT	RTFSHASDTW	MFGVTLWEMF	TYGQEPWIGL	480
481	NGSQILHKID	KEGERLPRPE	DCPQDIYNVM	VQCWAHKPED	RPTFVALRDF	LLEAQPTDMR	540
541	ALQDFEEPDK	LHIQMN DVIT	VIEGRAENYW	WRGQNTRTL C	VGPFPRNVVT	SVAGLSAQDI	600
601	SQPLQNSFIH	TGHGSDPRH	CW				660

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:** ACK1 fragment

ACK1 wt ¹ Amino Acid Sequence							
1	MQPEEGTGWL	LELLSEVQLQ	QYFLRLRDDL	NVTRL SHFEY	VKNEDLEKIG	MGRPGQRR LW	60
61	EAVKRRKALC	KRKSWSKV F	SGKRLEAEFP	PHHSQSTFRK	TSPAPGGPAG	EGPLQSLTCL	120
121	IGEKDLR LLE	KLGDGSFGVV	RRGEWDAPSG	KTVSVAVKCL	KPDVLSQPEA	MDDFIREVNA	180
181	MHSLDHRNLI	RLYG VVLTTP	MKMVTE LAPL	GSLLDRLRKH	QGHFLLG TLS	RYAVQVAEGM	240
241	GYLESKRFIH	RDLAARNLLL	ATRD LVKIGD	FGLMRALPQN	DDHYVMQ EHR	KVPFAWCAPE	300
301	SLKTRTFSHA	SDTWMFGVTL	WEMFTYQEP	WIGLNGSQIL	HKIDKEGERL	PRPEDCPQDI	360
361	YNVMVQCWAH	KPEDRPTFVA	LRDFLLEAQP	TDMRALQDFE	EPDKLHIQMN	DVITVIEGRA	420
421	ENYWWRGQNT	RTL CVGPFPR	NVTVSVAGLS	AQDISQPLQN	SFIHTGHGDS	DPRHCW GFPD	480
481	RIDELYLGNP	MDPPDLLSVE	LSTSRPPQHL	GGVKKPTYDP	VSEDQDPLSS	DFKRLGLRKP	540
541	GLPRGLWLAK	PSARVPGTKA	SRGSGAEVTL	IDFGEEP VVP	ALRPCAPSLA	QLAMDACSL L	600
601	DETPPQS PTR	ALPRPLHPTP	VVDWDARPLP	PPPAYDDVAQ	DEDDFEICSI	NSTLVGAGVP	660
661	AGPSQGQ TNY	AFVPEQARPP	PLEDNLFLP	PQGGGKPPSS	AQTAEIFQAL	QQECMRQLQA	720
721	PAGSPAPSPS	PGGDDKPQVP	PRVPIPPRPT	RPHVQLSPAP	PGEEETSQWP	GPASPPRVPP	780
781	REPLSPQGS R	TPSPLVPPGS	SPLPPRLSSS	PGKTMPTTQS	FASDPKYATP	QVIQAPGPRA	840
841	GPCILPIVRD	GKKVSSTHYY	LLPERPSYLE	RYQRFLREAQ	SPEEPTPLPV	PLLLPPPSTP	900
901	APAAPTATVR	PMPQAALDPK	ANFSTNNSNP	GARPPPPRAT	ARLPQRGCPG	DGPEAGRPAD	960
961	KIQMAMVHGV	TTEECQAALQ	CHGWSVQRAA	QYLKVEQLFG	LGLRPRGECH	KVLEMFDWNL	1020
1021	EQAGCHLLGS	WGPAHHR					1080

blue: ACK1 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_005772.3