

Certificate of Analysis



Jak1 aa583-1154 S729C

Janus kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: JAK1

Synonyms: JAK1A, JAK1B, JTK3

Product No.: 1481-0000-1

Lot: 002

Description: Human JAK1, C-terminal fragment, amino acids L₅₈₃-K₁₁₅₄ (as in NCBI/Protein entry NP_002218.2) with a S729C mutation, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: JAK1 aa583-1154 S729C Lot 002, was confirmed as JAK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 93,643 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

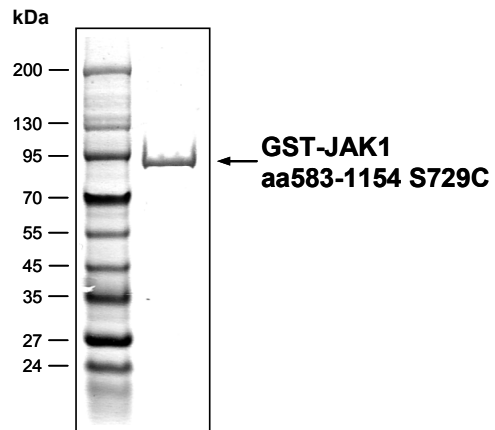
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.285 µg/µl (Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

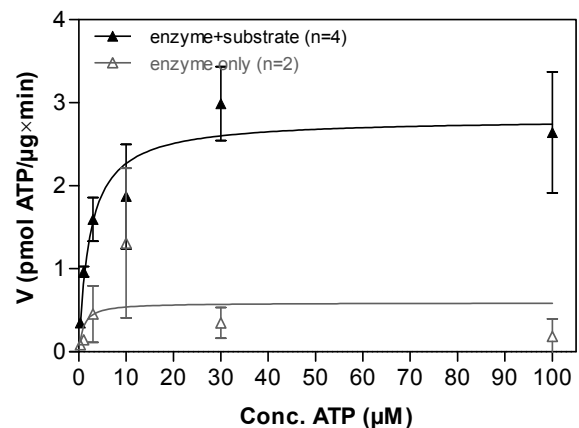
Specific kinase activity (P_i transfer): 2.8 pmol/µg×min
ATP-K_M: 2.4 µM

**JAK1 aa583-1154 S729C Lot 002:
Coomassie stain**



2.0 µg GST-JAK1


**JAK1 wt aa583-1154 S729C 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: RBER-IRStide, 80 µg / ml
 - JAK1 S729C aa583-1154 Lot 002: 1.0 µg / ml
- Filter binding assay
- MSFC membrane (Millipore)

Additional assay technology: JAK1 aa583-1154 S729C Lot 002

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

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JAK1 aa583-1154 S729C

Product No.: 1481-0000-0

JAK1 aa583-1154 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RDSLEVLFGQ	240
241	PLVQGEHLGR	GTRTHIYSGT	LMDYKDDEGT	SEEKIKIVIL	KVLDPSHRDI	SLAFFEAASM	300
301	MRQVSHKHIV	YLYGVCVRDV	ENIMVEEFVE	GGPLDLFMHR	KSDVLTTPWK	FKVAKQLASA	360
361	LSYLEDKDLV	HGNVCTKNLL	LAREGIDCEC	GPFIKLSDPG	IPITVLSRQE	CIERIPWIAP	420
421	ECVEDSKNLS	VAADKWSFGT	TLWEICYNGE	IPLKDKTLIE	KERFYESRCR	PVTPSCKELA	480
481	DLMTRCMNYD	PNQRPFRAI	MRDINKLEEQ	NPDIVSEKKP	ATEVDPTHFE	KRFLKRIRDL	540
541	GEGHFGKVEL	CRYDPEGDNT	GEQVAVKSLK	PESGGNHIAD	LKKEIEILRN	LYHENIVKYK	600
601	GICTEDGGNG	IKLIMEFLPS	GSLKEYLPKN	KNKINLKQQL	KYAVQICKGM	DYLGSRQYVH	660
661	RDLAARNVLV	ESEHQVKIGD	FGLTKAIETD	KEYYTVKDDR	DSPVFWYAPE	CLMQSKFYIA	720
721	SDVWSFGVTL	HELLTYCDS	SSPMALFLKM	IGPTHGQMTV	TRLVNTLKEG	KRLPCPPNCP	780
781	DEVYQLMRKC	WEFQPSNRTS	FQNLIEGFEA	LLK			840

1-218: GST **Red:** HIS6-tag **Green:** 3C cleavage site **blue:**JAK1 fragment **boxed:** S729C

JAK1 wt ¹ Amino Acid Sequence							
1	MQYLNIEDC	NAMAFCAKMR	SSKKTEVNLE	APEPGVEVIF	YLSDREPLRL	GSGEYTAEEEL	60
61	CIRAAQACRI	SPLCHNLFAL	YDENTKLWYA	PNRTITVDDK	MSLRLHYRMR	FYFTNWHGTN	120
121	DNEQSVWRHS	PKKQKNGYEK	KKIPDATPLL	DASSLEYLFA	QGQYDLVKCL	APIRDPKTEQ	180
181	DGHDIENECL	GMAVLAISHY	AMMKKMLPE	LPKDISYKRY	IPETLNKSIR	QRNLLTRMRI	240
241	NNVFKDFLKE	FNNKTICDSS	VSTHDLKVYK	LATLETLTKH	YGAEIFETSM	LLISSENMEN	300
301	WFHSDNGGNV	LYYEVMTGN	LGIQWRHKPN	VVSVEKEKNK	LKRKKLENKH	KKDEEKNKIR	360
361	EEWNNFSYFP	EITHIVIKES	VVSINKQDNK	KMELKLSSHE	EALSFVSLVD	GYFRLTADAH	420
421	HYLCTDVAPP	LIVHNIQNGC	HGPICTEYAI	NKLRQEGSEE	GMVVLRSWCT	DFDNILMTVT	480
481	CFEKSEQVQG	AQKQFKNFQI	EVQKGRYSLH	GSDRSFPSLG	DLMSHLKKQI	LRTDNISFML	540
541	KRCCQPKPRE	ISNLLVATKK	AQEWQPVYPM	SQLSFDRIK	KDLVQGEHLG	RGTRTHIYSG	600
600	TLMDYKDDEG	TSEEKIKIVI	LKVLDPSHRD	ISLAFFEAAS	MMRQVSHKHI	VYLYGVCVRD	660
661	VENIMVEEFV	EGGPLDLFMH	RKSDVLTTPW	KFKVAKQLAS	ALSYLEDKDL	VHGNVCTKNL	720
721	LLAREGIDSE	CGPFIKLSDP	GIPITVLSRQ	ECIERIPWIA	PECVEDSKNL	SVAADKWSFG	780
781	TTLWEICYNG	EIPLKDKTLI	EKERFYESRC	RPVTPSCKEL	ADLMTRCMNY	DPNQRPFRAI	840
841	IMRDINKLEE	QNPDIVSEKK	PATEVDPTHF	EKRFLKRIRD	LGEGHFGKVE	LCRYDPEGDN	900
901	TGEQVAVKSL	KPESGGNHIA	DLKKEIEILR	NLYHENIVKY	KGICTEDGGN	GIKLIMEFLP	960
961	SGSLKEYLPK	NKNKINLKQ	LKYAVQICKG	MDYLGSRQYV	HRDLAARNVL	VESEHQVKIG	1020
1021	DFGLTKAIET	DKEYYTVKDD	RDSPVFWYAP	ECLMQSKFYI	ASDVWSFGVT	LHELLTYCDS	1080
1081	DSSPMALFLK	MIGPTHGQMT	VTRLVNTLKE	GKRLPCPPNC	PDEVYQLMRK	CWEFQPSNRT	1140
1141	SFQNLIEGFE	ALLK					1200

blue: JAK1 sequence expressed in fusionprotein **RED:** variant in fusion protein

¹NCBI/Protein accession number NP_002218.2

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