

Certificate of Analysis



ABL1 F317I

v-abl Abelson murine leukemia viral oncogene homolog 1

Recombinant Human Active Protein Kinase

Synonyms: c-Abl, JTK7, p150

Product No.: 0989-0000-1

Lot: 001

Description: Human ABL1, internal fragment, amino acids P₁₁₈-S₅₃₅ (as in GenBank entry NM_005157.3), F317I mutant, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: ABL1 F317I Lot 001 was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 76,133 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

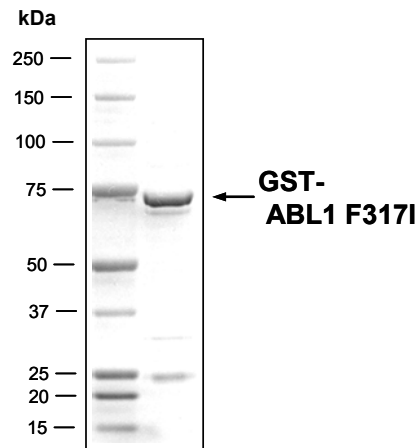
Protein concentration: 0.121 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

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(Ala,Glu,Lys,Tyr)_{6:2:5:1} (Sigma P-1152), 20 µg / ml
 - ABL1 F317I: 2.0 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

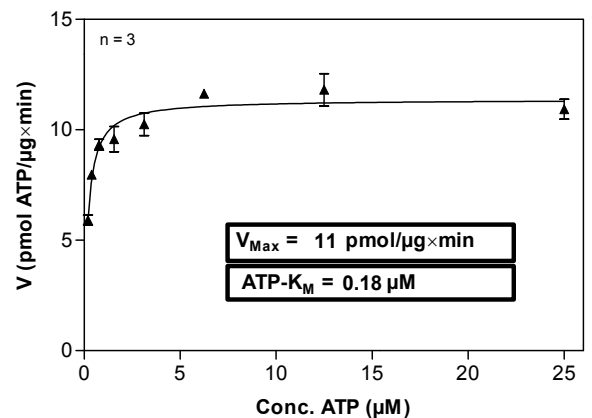
Specific activity: 11 pmol/µg×min

ABL1 F317I Lot 001: Coomassie stain



2,0 µg GST-ABL1 F317I

ABL1 F317I Lot 001: Determination of V_{max} and K_m value for ATP



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ABL1 F317I Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGQ	240
241	PLAMLVNSL	EKHSWYHGPV	SRNAAEYLLS	SGINGSFLVR	ESESSPGQRS	ISLRYEGRVY	300
301	HYRINTASDG	KLYVSSESFR	NLAELVHHH	STVADGLITT	LHYPAPKRNK	PTVYGVSPNY	360
361	DKWEMERTDI	TMKHKLGGGQ	YGEVYEGVWK	KYSLTVAVKT	LKEDTMEVEE	FLKEAAMKE	420
421	IKHPNLVQLL	GVCTREPPFY	IITEIMTYGN	LLDYLRECNR	QEVNAVLLY	MATQISSAME	480
481	YLEKKNFIHR	DLAARNCLVG	ENHLVKVADF	GLSRLMTGDT	YTAHAGAKFP	IKWTAPESLA	540
541	YNKFSIKSDV	WAFGVLLWEI	ATYGMSPYPG	IDLSDQVYELL	EKDYRMERPE	GCPEKVYELM	600
600	RACQWNPSPD	RPSFAEIQHA	FETMFQESSI	SDEVEKELGK	QGVRGAVSTL	LQAPPELPTKT	660
661	RTS						720

1-218: GST Red: HIS6-tag Green: 3C blue: ABL1 fragment I: F317I mutation

ABL1 wt ORF (taken from GenBank entry NM_005157.3)							
1	MLEICLKLVG	CKSKKGLSSS	SSCYLEEALQ	RPVASDFEPQ	GLSEAARWNS	KENLLAGPSE	60
61	NDPNLFFVALY	DFVASGDNTL	SITKGEKLRV	LGYNHNGEWC	EAQTKNGQGW	VPSNYITPVN	120
121	SLEKHSWYHG	PVSRNAEYL	LSSGINGSFL	VRESESSPGQ	RSISLRYEGR	VYHYRINTAS	180
181	DGKLYVSSES	RFNTLAELVH	HHSTVADGLI	TTLHYPAPKR	NKPTVYGVSP	NYDKWEMERT	240
241	DITMKHKLGG	GQYGEVYEGV	WKKYSLTVAV	KTLKEDTMEV	EEFLKEAAM	KEIKHPNLVQ	300
301	LLGVCTREPP	FYIITEFMTY	GNLLDYLREC	NRQEVNAVVL	LYMATQISSA	MEYLEKKNFI	360
361	HRDLAARNCL	VGENHLVKVA	DFGLSRLMTG	DTYTAHAGAK	FPIKWTAPES	LAYNKFSIKS	420
421	DVWAFGVLLW	EIATYGMSPY	PGIDLSDQVYE	LLEKDYRMER	PEGCPEKVYE	LMRACWQWNP	480
481	SDRPSFAEIH	QAFETMFQES	SISDEVEKEL	GKQGVRGAVS	TLLQAPPELPT	KTRTSRRAAE	540
541	HRDITDVPPEM	PHSKQGQESD	PLDHEPAVSP	LLPRKERGPP	EGGLNEDERL	LPKDKKTNLF	600
600	SALIKKKKKT	APTPPKRSSS	FREMDGQPER	RGAGEEEGRD	ISNGALAFPT	LDTADPAKSP	660
661	KPSNGAGVPN	GALRESGGSG	FRSPHLWKKS	STLTSSRLAT	GEEEGGGSSS	KRFLRSCSAS	720
721	CVPHGAKDTE	WRSVTLPRDL	QSTGRQFDSS	TFGGHKSEKP	ALPRKRAGEN	RSDQVTRGTV	780
781	TPPPRLVKKN	EAADEVFKD	IMESSPGSSP	PNLTPKPLRR	QVTVPASGL	PHKEEAGKGS	840
841	ALGTPAAAEF	VTPTSKAGSG	APGGTSKGPA	EESRVRRHKH	SSESPGRDKG	KLSRLKPAPP	900
901	PPPAASAGKA	GGKPSQSPSQ	EAAGEAVLGA	KTKATSLVDA	VNSDAAKPSQ	PGEGLKKPVL	960
961	PATPKPQSAK	PSGTPISPAP	VPSTLPSASS	ALAGDQPSST	AFIPLISTRV	SLRKTRQPPE	1020
1021	RIASGAITKG	VVLDSTEALC	LAISRNSEQM	ASHSAVLEAG	KNLYTFCVSY	VDSIQQMRNK	1080
1081	FAFREAINKL	ENNLRELQIC	PATAGSGPAA	TQDFSKLLSS	VKEISDIVQR		1140

blue: fragment of ABL1 expressed in fusionprotein RED: F317I mutation site

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