Certificate of Analysis

ProQinase™ T315I

ABL proto-oncogene 1, non-receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: ABL1

Synonyms: ABL, c-Abl, JTK7, p150

Product No.: 0757-0000-1

Lot: 003

Description: Human ABL1, internal fragment, amino acids P_{118}-S_{535} (as in NCBI/Protein entry NP_005148.2), T315I mutant, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: ABL1 T315I Lot 003 was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical MW$_{\text{Fusion Protein}}$: 76,179 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

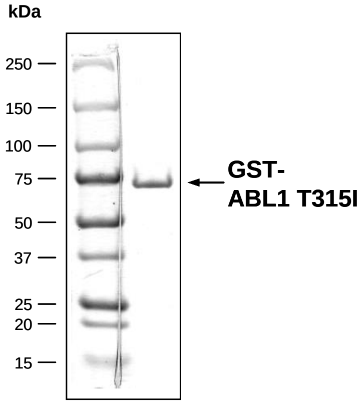
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

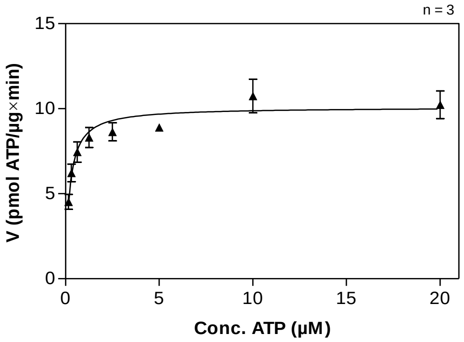
Biochemical Parameters:
Specific kinase activity (P_i transfer): 10 pmol/µg × min
ATP-K_M: 0.21 µM

ABL1 T315I Lot 003: Coomassie stain



2.0 µg GST-ABL1 T315I

ABL1 T315I 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 $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: Poly(Ala,Glu,Lys,Tyr)$_{6:2:5:1}$, 20 µg/ml
  ABL1 T315I: 2 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Recombinant Proteins

Additional assay technology:
ABL1 T315I Lot 003 was also successfully tested by Reaction Biology 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

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GST-ABL1 T315I 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	KRIEAIPQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG**HHHHHH**G	RRRASVAAGI	240
241	**LVPRGS**PGLD	GIYARDS**LEV**	**LFQGP**LAML**P**	**VNSLEKHSWY**	**HGPVSRNAAE**	**YLLSSGINGS**	300
301	**FLVRESESSP**	**GQRSISLRYE**	**GRVYHYRINT**	**ASDGKLYVSS**	**ESRFNTLAEL**	**VHHHSTVADG**	360
361	**LITTLHYPAP**	**KRNKPTVYGV**	**SPNYDKWEME**	**RTDITMKHKL**	**GGGQYGEVYE**	**GVWKKYSLTV**	420
421	**AVKTLKEDTM**	**EVEEFLKEAA**	**VMKEIKHPNL**	**VQLLGVCTRE**	**PPFYIIIEFM**	**TYGNLLDYLR**	480
481	**ECNRQEVNAV**	**VLLYMATQIS**	**SAMEYLEKKN**	**FIHRDLAARN**	**CLVGENHLVK**	**VADFGLSRLM**	540
541	**TGDTYTAHAG**	**AKFPIKWTAP**	**ESLAYNKFSI**	**KSDVWAFGVL**	**LWEIATYGMS**	**PYPGIDLSQV**	600
600	**YELLEKDYRM**	**ERPEGCPEKV**	**YELMRACWQW**	**NPSDRPSFAE**	**IHQAFETMFQ**	**ESSISDEVEK**	660
661	**ELGKQGVRGA**	**VSTLLQAPEL**	**PTKTRTS**				720

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **Green**: 3C cleavage site **blue**: ABL1 fragment **boxed**: T315I mutation

ABL1 wt[1] Amino Acid Sequence

1	MLEICLKLVG	CKSKKGLSSS	SSCYLEEALQ	RPVASDFEPQ	GLSEAARWNS	KENLLAGPSE	60
61	NDPNLFVALY	DFVASGDNTL	SITKGEKLRV	LGYNHNGEWC	EAQTKNGQGW	VPSNYIT**PVN**	120
121	**SLEKHSWYHG**	**PVSRNAAEYL**	**LSSGINGSFL**	**VRESESSPGQ**	**RSISLRYEGR**	**VYHYRINTAS**	180
181	**DGKLYVSSES**	**RFNTLAELVH**	**HHSTVADGLI**	**TTLHYPAPKR**	**NKPTVYGVSP**	**NYDKWEMERT**	240
241	**DITMKHKLGG**	**GQYGEVYEGV**	**WKKYSLTVAV**	**KTLKEDTMEV**	**EEFLKEAAVM**	**KEIKHPNLVQ**	300
301	**LLGVCTREPP**	**FYIITEFMTY**	**GNLLDYLREC**	**NRQEVNAVVL**	**LYMATQISSA**	**MEYLEKKNFI**	360
361	**HRDLAARNCL**	**VGENHLVKVA**	**DFGLSRLMTG**	**DTYTAHAGAK**	**FPIKWTAPES**	**LAYNKFSIKS**	420
421	**DVWAFGVLLW**	**EIATYGMSPY**	**PGIDLSQVYE**	**LLEKDYRMER**	**PEGCPEKVYE**	**LMRACWQWNP**	480
481	**SDRPSFAEIH**	**QAFETMFQES**	**SISDEVEKEL**	**GKQGVRGAVS**	**TLLQAPELPT**	**KTRTS**RRAAE	540
541	HRDTTDVPEM	PHSKGQGESD	PLDHEPAVSP	LLPRKERGPP	EGGLNEDERL	LPKDKKTNLF	600
600	SALIKKKKKT	APTPPKRSSS	FREMDGQPER	RGAGEEEGRD	ISNGALAFTP	LDTADPAKSP	660
661	KPSNGAGVPN	GALRESGGSG	FRSPHLWKKS	STLTSSRLAT	GEEEGGGSSS	KRFLRSCSAS	720
721	CVPHGAKDTE	WRSVTLPRDL	QSTGRQFDSS	TFGGHKSEKP	ALPRKRAGEN	RSDQVTRGTV	780
781	TPPPRLVKKN	EEAADEVFKD	IMESSPGSSP	PNLTPKPLRR	QVTVAPASGL	PHKEEAGKGS	840
841	ALGTPAAAEP	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: ABL1 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1] NCBI/Protein accession number NP_005148.2

Recombinant Proteins