

Certificate of Analysis

Abl (M351T), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-757, 14-757-K, 14-757M

Parent Lot # 1643914

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged recombinant human Abl, residues 27–end containing the M351T mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose.

The Abl tyrosine kinase inhibitor STI571 is an effective therapy for stable-phase chronic myeloid leukaemia patients. Many patients responding to STI571 later relapse due to a reactivation of Bcr-Abl activity. In certain cases this appears to correlate with the presence of the M351T mutation, which confers resistance to drug binding (Shah et al., (2002), Cancer Cell 2, 117-125).

Purity 40% by SDS-PAGE and Coomassie staining. MW = 121.4kDa.

Formulation: 0.783mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

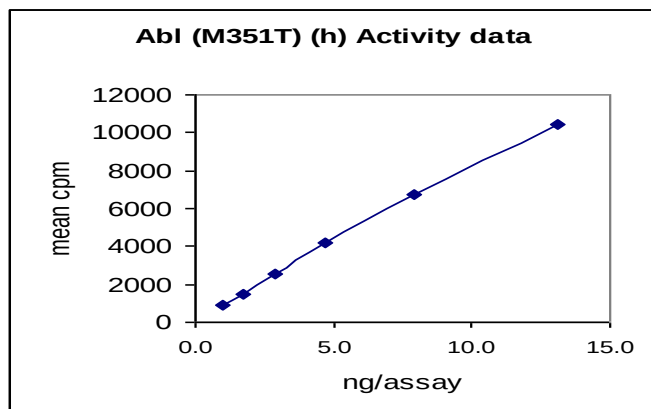
Specific Activity (Parent lot# 1643914): 899U/mg, where one unit of Abl (M351T), active activity is defined as 1nmol phosphate incorporated into 50µM Abltide (EAIYAAPFAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

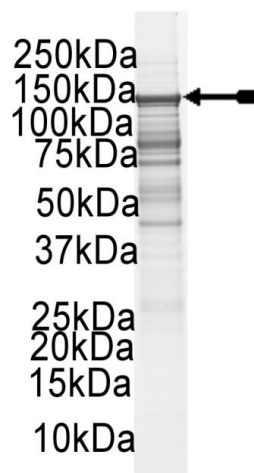
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.0–13.1ng of this lot of enzyme phosphorylated 50µM Abltide (EAIYAAPFAKKK) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as Abl (M351T) with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Abl (M351T), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Abltide (EAIYAAPFAKKK):** Use at a final assay concentration of 50µM. Prepare a 500µM stock and add 2.5µl of stock per assay point.
3. **Abl (M351T), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.0–13.1ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **Abltide**.
3. Add **2.5µl (1.0–13.1ng Abl (M351T), active)**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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Abl (M351T) Sequence Information

<u>Protein</u>	Human Abl (27–end, M351T)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	E10 of recombinant sequence is equivalent to E27 of native human Abl
<u>Accession number</u>	GenBank U07563

Recombinant Abl (M351T) amino acid sequence:

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1  MHHHHHHEFE  ALQRPVASDF  EPQGLSEAR  WNSKENLLAG  PSENDPNLFV  ALYDFVASGD
61  NTLISITKGEK  LRVLGYNHNG  EWCEAQTKNG  QGWVPSNYIT  PVNSLEKHSW  YHGPVSRNAA
121 EYLLSSGING  SFLVRESESS  PGQRSISLRY  EGRVYHYRIN  TASDGKLYVS  SESRFNTLAE
181 LVHHHSTVAD  GLITTLHYPA  PKRNKPTVYG  VSPNYDKWEM  ERTDITMKHK  LGGGQYGEVY
241 EGVWKYSLT  VAVKTLKEDT  MEVEEFLKEA  AVMKEIKHPN  LVQLLGVCTR  EPPFYIITEF
301 MTYGNLLDYL  RECNRQEVNA  VVLLYMATQI  SSATEYLEKK  NFIHRDLAAR  NCLVGENHLV
361 KVADFGLSRL  MTGDTYTAHA  GAKFPIKWT  PESLAYNKFS  IKSDVWAFGV  LLWEIATYGM
421 SPYPGIDLSQ  VYELLEKDYR  MERPEGCPEK  VYELMRACWQ  WNPSDRPSFA  EIHQAFETMF
481 QESSISDEVE  KELGKQGVRG  AVSTLLQAPE  LPTKTRTSRR  AAEHRDSTDV  PEMPHSKGQG
541 ESDPLDHEPA  VSPLLPRKER  GPPEGGLNED  ERLLPKDKKT  NLFSALIKKK  KKTAPTTPKR
601 SSSFREMDGQ  PERRGAGEEE  GRDISNGALA  FTPLDTADPA  KSPKPSNGAG  VPNGALRESG
661 GSGFRSPHLW  KKSSTLTSSR  LATGEEEGGG  SSKRFLRSC  SASCVPHGAK  DTEWRSVTLF
721 RDLQSTGRQF  DSSTFGGHKS  EKPALPRKRA  GENRSDQVTR  GTVTPPPRLV  KKNEEADEV
781 FKDIMESSPG  SSPPNLTPKP  LRRQVTVAPA  SGLPHKEEAG  KGSALGTPAA  AEPVTPTSKA
841 GSGAPGGTSK  GPAEESRVRR  HKHSSESPPR  DKGKLSRLKP  APPPPPAASA  GKAGGKPSQS
901 PSQEAAGEAV  LGAKTKATSL  VDAVNSDAAK  PSQPG EGLKK  PVLPATPKPQ  SAKPSGTPIS
961 PAPVPSTLPS  ASSALAGDQP  SSTAFIPLIS  TRVSLRKTRQ  PPERIASGAI  TKGVLVDSTE
1021 ALCLAISRNS  EQMASHAVL  EAGKNLYTFC  VSYVDSIQQM  RNKF AFREAI  NKLENNLREL
1081 QICPATAGSG  PAATQDFSKL  LSSVKEISDI  VQR
  
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Recombinant Abl (M351T) nucleotide sequence:

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1  atgcatcatc  accatcacca  tgaattcgaa  gcccttcagc  ggccagtagc  atctgacttt
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121 cccagtgaaa  atgaccccaa  ctttttcgtt  gcactgtatg  attttgtggc  cagtggagat
181 aacactctaa  gcataactaa  aggtgaaaag  ctccgggtct  taggctataa  tcacaatggg
241 gaatggtgtg  aagcccaaac  caaaaatggc  caaggctggg  tccaagcaa  ctacatcacg
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421 cctggccaga  ggtccatctc  gctgagatac  gaaggagggg  tgtaccatta  cagatcaaac
481 actgcttctg  atggcaagct  ctacgtctcc  tccgagagcc  gcttcaaac  cctggccgag
541 ttggttcac  atcattcaac  ggtggccgac  gggctcatca  ccacgctcca  ttatccagcc
601 ccaaagcgca  acaagcccac  tgtctatggt  gtgtcccca  actacgacaa  gtgggagatg
661 gaacgcacgg  acatcaccat  gaagcacaag  ctgggcgggg  gccagtagcg  ggaggtgtac
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781 atggaggtgg  aagagttctt  gaaagaagct  gcagtcatga  aagagatcaa  acaccctaac
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1381 tggaatccct ctgaccggcc ctcccttctgct gaaatccacc aagcctttga aacaatgttc
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