

Certificate of Analysis

Abl (T315I), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-522, 14-522-K, 14-522M

Parent Lot # 33235U

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 T315I mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 27% by SDS-PAGE and Coomassie staining. MW = 121.4kDa.

Specific Activity (Parent lot# 33235U): 743U/mg, where one unit of Abl (T315I), active activity is defined as 1nmol phosphate incorporated into 50µM Abltide (EAIYAAPFAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.948mg/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.

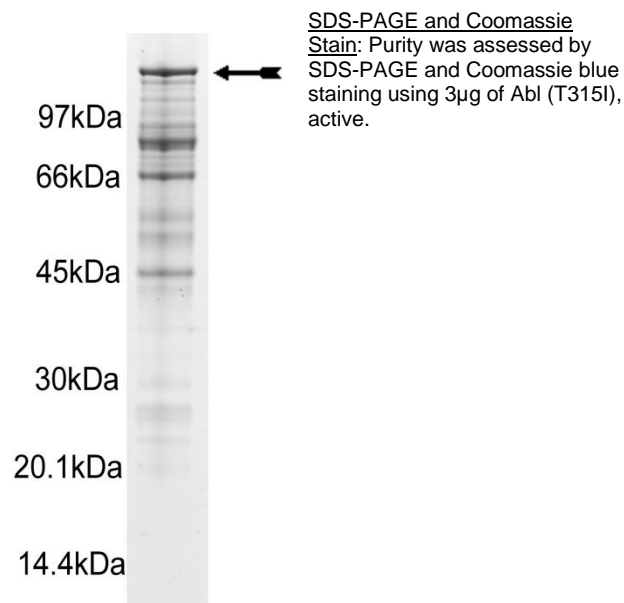
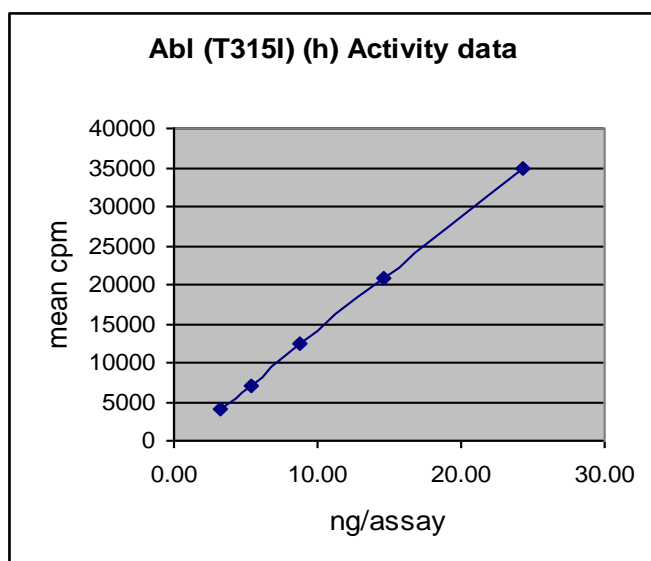
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 3–24ng of this lot of enzyme phosphorylated 50µM Abltide 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 identity as Abl (T315I) with the translated sequence listed on page three.



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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 50 μ M. Prepare a 500 μ M stock and add 2.5 μ l of stock per assay point.
3. **Abl (T315I), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3–24ng 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 5 μ l of 5 x reaction buffer.
2. Add 2.5 μ l of **(EAIYAAPFAKKK)**.
3. Add **2.5 μ l (3–24ng) Abl, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3 % phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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Abl (T315I) Sequence Information

<u>Protein</u>	Human Abl (27–end, T315I)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	E10 of the recombinant protein is equivalent to E27 of human Abl
<u>Accession number</u>	GenBank U07563. The recombinant protein contains the amino acid substitution, T315I with reference to U07563. This mutation is reported to elicit resistance to the Abl tyrosine kinase inhibitors STI-571.

Recombinant Abl (T315I) amino acid sequence:

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1 MHHHHHHEFE ALQRPVASDF EPQGLSEAAW WNSKENLLAG PSENDPNLFV ALYDFVASGD
61 NTL SITKGEK LRV LGYNHNG EWCEAQT KNG QGWVPSNYIT PVNSLEKHSW YHG PVS RNAA
121 EYLLSSGING SFLVRESESS PGQRSISLRY EGRVYHYRIN TASDGKLYVS SESRFNTLAE
181 LVHHHSTVAD GLITTLHYPA PKRNKPTVYG VSPNYDKWEM ERTDITMKHK LGGGQYGEVY
241 EGVWKKYSLT VAVKTLKEDT MEVEEFLKEA AVMKEIKHPN LVQLLGVCTR EPPFYIIIEF
301 MTYGNLLDYL RECNRQEVNA VVLLYMATQI SSAMEYLEKK NFIHRDLAAR NCLVGENHLV
361 KVA DFGLSRL MTGD TYTAHA GAKFPIK WTA PESLAYNKFS IKSDVWAFGV LLWEIATYGM
421 SPYPGIDLSQ VYELLEKDYR MERPEGCPEK VYELMRACWQ WNP SDRPSFA EIHQAFETMF
481 QESSISDEVE KELGKQGV RG AVSTLLQAPE LPTKTRTSRR AAEHRD TTDV PEMPHSKGQG
541 ESDPLDHEPA VSPLLPRKER GPPEGGLNED ERLLPKDKKT NLF SALIKKK KKTAPT PPKR
601 SSSFREMDGQ PERRGAGEEE GRDISGNALA FTPLDTADPA KSPKPSNGAG VPNGALRESG
661 GSGFRSPHLW KKSSTLTSSR LATGEEEGGG SSSKRFLRSC SASCVPHGAK DTEWRSVTLP
721 RDLQSTGRQF DSSTFGGHKS EKPALPRKRA GENRSDQVTR GTVT P P PRLV KKNEEADEV
781 FKDIMESSPG SSPPNLTPKP LRRQVTVAPA SGLPHKEEAG KGSALGTPAA AEPV T P TSKA
841 GSGAPGGTSK GPAEESRVR HKHSSESPGR DKGKLSRLKP A P P P P P AASA GKAGGKPSQS
901 PSQEAAGEAV LGAKTKATSL VDAVNSDAAK PSQPGEGLKK PVL PAT PKPQ SAKPSGTPIS
961 PAPVPSTLPS ASSALAGDQP SSTAFIPLIS TRVSLRKRTRQ PPERIASGAI TKGVVLDSTE
1021 ALCLAISRNS EQMASHSAVL EAGKNLYTFC VSYVDSIQQM RNKF AFREAI NKLENNLREL
1081 QICPATAGSG PAATQDFSKL LSSVKEISDI VQR

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Recombinant Abl (T315I) nucleotide sequence:

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1 atgcatcatc accatcacca tgaattcgaa gcccttcagc ggccagtagc atctgacttt
61 gagcctcagg gtctgagtga agccgctcgt tggaaactcca agggaaacct tctcgetgga
121 cccagtgaaa atgaccccaa ctttttcgtt gcactgtatg attttgtggc cagtggagat
181 aacactctaa gcataactaa aggtgaaaag ctccgggtct taggctataa tcacaatggg
241 gaatggtgtg aagcccaaac caaaaatggc caaggctggg tccaagcaa ctacatcacg
301 ccagtcaaca gtctggagaa acactcctgg taccatgggc ctgtgtcccg caatgccgct
361 gagtatctgc tgcagcgcg gatcaatggc agcttcttgg tgcgtgagag tgagagcagt
421 cctggccaga ggtccatctc gctgagatac gaagggaggg tgtaccatta caggatcaac
481 actgcttctg atggcaagct ctacgtctcc tccgagagcc gcttcaacac cctggccgag
541 ttggttcatc atcattcaac ggtggccgac gggctcatca ccacgetcca ttatccagcc
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