

Certificate of Analysis

Abl (Q252H), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-751, 14-751-K, 14-751M

Parent Lot # D7DN038U

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 Q252H 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 Q252H mutation, which confers resistance to drug binding (Shah et al., (2002), Cancer Cell 2, 117-125). Purity 52% by SDS-PAGE and Coomassie staining. MW = 121kDa.

Specific Activity (Parent lot# D7DN038U): 1127U/mg, where one unit of Abl (Q252H), 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: 2.044mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 270mM sucrose, 1mMbenzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

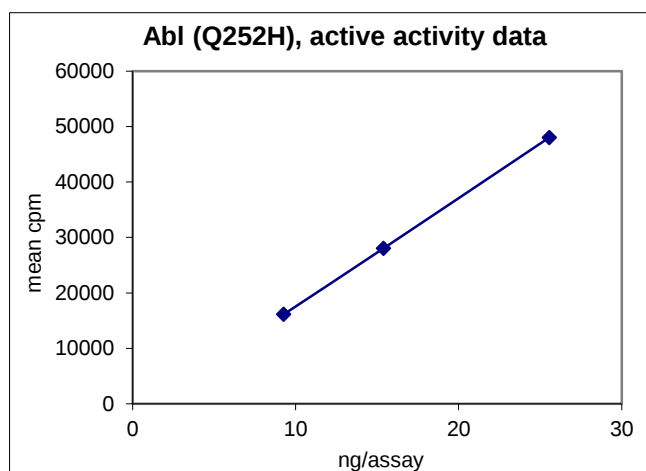
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

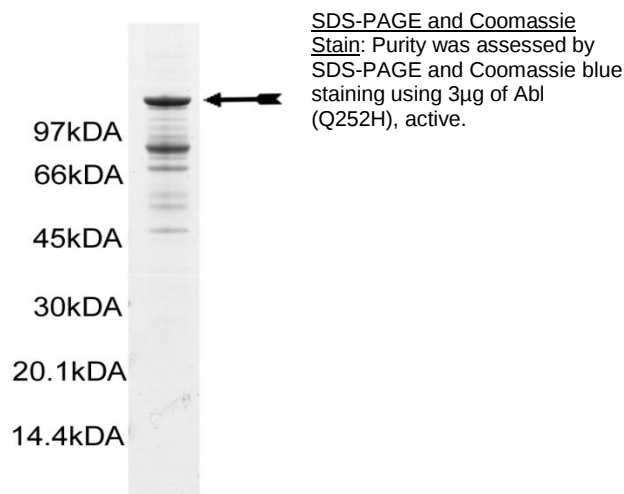
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 9–25ng 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 (Q252H) with the translated sequence listed on page three.



Certificate of Analysis

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 (Q252H), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9–25ng 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 (9–25ng) Abl (Q252H), 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.

Certificate of Analysis

Abl (Q252H) Sequence Information

<u>Protein</u>	Human Abl (27–end, Q252H)
<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 (Q252H) amino acid sequence:

```

1  MHHHHHHEFE  ALQRPVASDF  EPQGLSEAR  WNSKENLLAG  PSENDPNLFV  ALYDFVASGD
61  NTLSTITKGEK  LRLVGYNHNG  EWCEAQTKNG  QGWVPSNYIT  PVNSLEKHSW  YHGPVSRNAA
121 EYLLSSGING  SFLVRESESS  PGQRSISLRY  EGRVYHYRIN  TASDGKLYVS  SESRFNTLAE
181 LVHHHSTVAD  GLITTLHYPA  PKRNKPTVYG  VSPNYDKWEM  ERTDITMKHK  LGGGHYGEVY
241 EGVWKYSLT  VAVKTLKEDT  MEVEEFLKEA  AVMKEIKHPN  LVQLLGVCTR  EPPFYIITEF
301 MTYGNLLDYL  RECNRQEVNA  VLLYMATQI  SSAMEYLEKK  NFIHRDLAAR  NCLVGENHLV
361 KVADFGLSRL  MTGDTYTAHA  GAKFPIKWT  PESLAYNKFS  IKSDVWAFGV  LLWEIATYGM
421 SPYPGIDLSQ  VYELLEKDYR  MERPEGCEPK  VYELMRACWQ  WNPSDRPSFA  EIHQAFETMF
481 QESSISDEVE  KELGKQGVRG  AVSTLLQAPE  LPTKTRTSRR  AAEHRDITDV  PEMPHSKGQG
541 ESDPLDHEPA  VSPLLPRKER  GPPEGGLNED  ERLLPKDKKT  NLFSALIKKK  KKTAPTPPKR
601 SSSFREMDGQ  PERRGAGEEE  GRDISNGALA  FTPLDTADPA  KSPKPSNGAG  VPNGALRESG
661 GSGFRSPHLW  KKSSTLTSSR  LATGEEEGGG  SSSKRFLRSC  SASCVPHGAK  DTEWRSVTLP
721 RDLQSTGRQF  DSSTFGGHKS  EKPALPRKRA  GENRSDQVTR  GTVTPPPRLV  KKNEEADEV
781 FKDIMESSPG  SSPNLTPKP  LRRQVTVAPA  SGLPHKEEAG  KGSALGTPAA  AEPVTPPSKA
841 GSGAPGGTSK  GPAESRVRR  HKHSSSESPGR  DKGKLSRLKP  APPPPPAASA  GKAGGKPSQS
901 PSQEAAGEAV  LGAKTKATSL  VDAVNSDAAK  PSQPGGLKK  PVLPAATPKPQ  SAKPSGTPIS
961 PAPVPSTLPS  ASSALAGDQP  SSTAFIPLIS  TRVSLRKTRQ  PPERIASGAI  TKGVVLDSTE
1021 ALCLAISRNS  EQMASHSAVL  EAGKNLYTFC  VSYVDSIQQM  RNKFAFREAI  NKLENNLREL
1081 QICPATAGSG  PAATQDFSKL  LSSVKEISDI  VQR
  
```

Recombinant Abl (Q252H) nucleotide sequence:

```

1  atgcatcatc  accatcacca  tgaattcgaa  gcccttcagc  ggccagtagc  atctgacttt
61  gagcctcagg  gtctgagtga  agccgctcgt  tggaactcca  aggaaaacct  tctcgtctga
121  ccagtgaaa  atgaccccaa  ccttttcgtt  gcaactgtatg  attttgtggc  cagtggagat
181  aacactctaa  gcataactaa  aggtgaaaag  ctccgggtct  taggctataa  tcacaatggg
241  gaatggtgtg  aagcccaaac  caaaaatggc  caaggctggg  tccaagcaa  ctacatcacg
301  ccagtcacaa  agctggagaa  acactcctgg  taccatgggc  ctgtgtcccg  caatgccgct
361  gagtatctgc  tgagcagcgg  gatcaatggc  agcttcttgg  tgcgtgagag  tgagagcagt
421  cctggccaga  ggtccatctc  gctgagatac  gaaggagggg  tgtaccatta  caggatcaac
481  actgcttctg  atggcaagct  ctacgtctcc  tccgagagcc  gcttcaacac  cctggccgag
541  ttggttcac  atcattcaac  ggtggccgac  gggctcatca  ccacgctcca  ttatccagcc
601  ccaaagcgca  acaagccac  tgtctatgg  gtgtcccca  actacgacaa  gtgggagatg
661  gaagcagcgg  acatcaccat  gaagcacaag  ctgggcgggg  gccactacgg  ggaggtgtac
721  gagggcgtgt  ggaagaaata  cagcctgacg  gtggccgtga  agaccttgaa  ggaggacacc
781  atggaggtgg  aagagttctt  gaaagaagct  gcagtcatga  aagagatcaa  acaccctaac
841  ctggtgcagc  tccttgggg  ctgcaccgg  gagccccgt  tctatatcat  cactgagttc
901  atgacctacg  ggaacctcct  ggactacctg  agggagtgc  accggcagga  ggtgaacgcc
961  gtggtgctgc  tgtacatggc  cactcagatc  tcgtcagcca  tggagtacct  ggagaagaaa
1021 aacttcatcc  acagagatct  tgctgccgga  aactgcctgg  taggggagaa  ccacttggtg
1081 aaggtagctg  attttggcct  gagcaggttg  atgacagggg  acacctacac  agcccatgct
1141 ggagccaagt  tccccatcaa  atggactgca  cccgagagcc  tggcctacaa  caagttctcc
1201 atcaagtccg  acgtctgggc  atttggagta  ttgctttggg  aaattgctac  ctatggcatg
  
```

Certificate of Analysis

```

1261 tcccccttacc cggaattga cctgtcccag gtgtatgagc tgctagagaa ggactaccgc
1321 atggagcgcc cagaaggctg cccagagaag gtctatgaac tcatgcgagc atgttggcag
1381 tggaatccct ctgaccggcc ctcttttgtc gaaatccacc aagcctttga aacaatgttc
1441 caggaatcca gtatctcaga cgaagtggaa aaggagctgg ggaaacaagg cgtccgtggg
1501 gctgtgagta ccttgctgca ggccccagag ctgcccacca agacaggagc ctccaggaga
1561 gctgcagagc acagagacac cactgacgtg cctgagatgc ctactccaa gggccaggga
1621 gagagcgatc ctctggacca tgagcctgcc gtgtctccat tgctccctcg aaaagagcga
1681 ggtcccccgaggggcggcct gaatgaagat gagcgccctc tccccaaaga caaaaagacc
1741 aacttgttca gcgccttgat caagaagaag aagaagacag cccaacccc tcccaaaccg
1801 agcagctcct tccgggagat ggacggccag ccggagcgca gaggggcccgg cgaggaagag
1861 ggccgagaca tcagcaacgg ggactggctc ttaccccctc tggacacagc tgaccagccc
1921 aagtccccaa agcccagcaa tggggctggg gtccccaatg gagccctccg ggagtccggg
1981 ggctcaggct tccggtctcc ccactgtgg aagaagtcca gcacgtgac cagcagctgc
2041 ctagccaccg gcgaggagga gggcggtggc agctccagca agcgcttcct gcgctcttgc
2101 tccgcctcct gcgttccccca tggggccaag gacacggagt ggaggtcagt cacgctgcct
2161 cgggacttgc agtccacggg aagacagttt gactcgtcca catttgaggg gcacaaaagt
2221 gagaagccgg ctctgcctcg gaagagggca ggggagaaca ggtctgacca ggtgaccgga
2281 ggcacagtaa cgctcccccc caggctggtg aaaaagaatg aggaagctgc tgatgaggtc
2341 ttcaaagaca tcatggagtc cagcccgggc tccagcccgc ccaacctgac tccaaaacc
2401 ctccggcggc aggtcacctg ggcccctgcc tcgggcctcc cccacaagga agaagctgga
2461 aagggcagtg ccttagggac ccctgctgca gctgagccag tgacccccac cagcaaagca
2521 ggctcaggtg caccaggggg caccagcaag ggcccgcgcg aggagtccag agtgaggagg
2581 cacaagcact cctctgagtc gccagggagg gacaagggga aattgtccag gctcaaacct
2641 gccccgcgc cccaccagc agcctctgca gggaaggctg gaggaaaagc ctgcagagc
2701 ccgagccagg agggcgccgg ggaggcagtc ctgggcgcaa agacaaaagc cacgagtctg
2761 gttgatgctg tgaacagtga cgctgccaag ccagccagc cgggagaggg cctcaaaaag
2821 cccgtgctcc cggccactcc aaagccacag tccgccaagc cgtcggggac ccccatcagc
2881 ccagcccccg ttccctccac gttgccatca gcacctctcg ccctggcagg ggaccagccg
2941 tcttccaccg ccttcatccc tctcatatca acccgagtgt ctcttcggaa aaccgcccag
3001 cctccagagc ggatcgccag cggcgccatc accaagggcg tggctcctgga cagcaccgag
3061 gcgctgtgcc tcgccatctc taggaactcc gagcagatgg ccagccacag cgcagtgtctg
3121 gaggccggca aaaacctcta cacgttctgc gtgagctatg tggattccat ccagcaaatg
3181 aggaacaagt ttgccttcg agaggccatc aacaaactgg agaataatct ccgggagctt
3241 cagatctgcc cggcgacagc aggcagtggg ccagcgcca ctcaggactt cagcaagctc
3301 ctcatgttcgg tgaaggaaat cagtgtatata gtgcagaggt ag

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services