eurofins | Discovery Services

Certificate of Analysis

Abl (H396P), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-750, 14-750-K, 14-750M
Parent Lot # D7BN009U

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 H396P mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose.
The Abl tyrosine kinase inhibitor STI571 (Gleevec™) 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 H396P mutation, which confers resistance to drug binding due to steric interference. (Corbin AS. *et al*, Blood, (2002); **101:4611**-4614 and La Rosee P. *et al*, Cancer Research; **62:7149**-7153).
Purity 23% by SDS-PAGE and Coomassie staining. MW = 121.4kDa.

Specific Activity (Parent lot# D7BN009U): 1790U/mg, where one unit of Abl (H396P), 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: 1.92mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

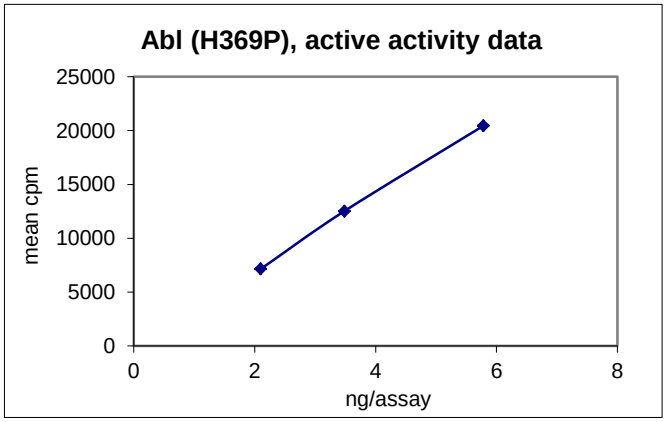
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

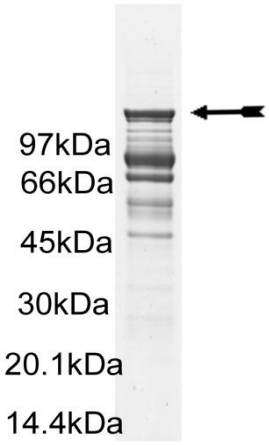
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2.1–5.8ng 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 (H396P) 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 (H396P), active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

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 (H396P), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.1–5.8ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 (2.1–5.8ng) Abl (H396P), active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P] 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.

eurofins | Discovery Services

Certificate of Analysis

Abl (H396P) Sequence Information

Protein Human Abl (27–end, H396P)

Tags *N*-terminal 6His

Native sequence E10 of the recombinant protein is equivalent to E27 of human Abl

Accession number GenBank U07563

Recombinant Abl (H396P) amino acid sequence:

```
   1 MHHHHHHEFE ALQRPVASDF EPQGLSEAAR WNSKENLLAG PSENDPNLFV ALYDFVASGD
  61 NTLSITKGEK LRVLGYNHNG EWCEAQTKNG QGWVPSNYIT PVNSLEKHSW YHGPVSRNAA
 121 EYLLSSGING SFLVRESESS PGQRSISLRY EGRVYHYRIN TASDGKLYVS SESRFNTLAE
 181 LVHHHSTVAD GLITTLHYPA PKRNKPTVYG VSPNYDKWEM ERTDITMKHK LGGGQYGEVY
 241 EGVWKKYSLT VAVKTLKEDT MEVEEFLKEA AVMKEIKHPN LVQLLGVCTR EPPFYIITEF
 301 MTYGNLLDYL RECNRQEVNA VVLLYMATQI SSAMEYLEKK NFIHRDLAAR NCLVGENHLV
 361 KVADFGLSRL MTGDTYTAPA GAKFPIKWTA PESLAYNKFS IKSDVWAFGV LLWEIATYGM
 421 SPYPGIDLSQ VYELLEKDYR MERPEGCPEK VYELMRACWQ WNPSDRPSFA EIHQAFETMF
 481 QESSISDEVE KELGKQGVRG AVSTLLQAPE LPTKTRTSRR AAEHRDTTDV 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 KKNEEAADEV
 781 FKDIMESSPG SSPPNLTPKP LRRQVTVAPA SGLPHKEEAG KGSALGTPAA AEPVTPTSKA
 841 GSGAPGGTSK GPAEESRVRR HKHSSESPGR DKGKLSRLKP APPPPPAASA GKAGGKPSQS
 901 PSQEAAGEAV LGAKTKATSL VDAVNSDAAK PSQPGEGLKK PVLPATPKPQ SAKPSGTPIS
 961 PAPVPSTLPS ASSALAGDQP SSTAFIPLIS TRVSLRKTRQ PPERIASGAI TKGVVLDSTE
1021 ALCLAISRNS EQMASHSAVL EAGKNLYTFC VSYVDSIQQM RNKFAFREAI NKLENNLREL
1081 QICPATAGSG PAATQDFSKL LSSVKEISDI VQR
```

Recombinant Abl (H396P) nucleotide sequence:

```
   1 atgcatcatc accatcacca tgaattcgaa gcccttcagc ggccagtagc atctgacttt
  61 gagcctcagg gtctgagtga agccgctcgt tggaactcca aggaaaacct tctcgctgga
 121 cccagtgaaa atgaccccaa ccttttcgtt gcactgtatg attttgtggc cagtggagat
 181 aacactctaa gcataactaa aggtgaaaag ctccgggtct taggctataa tcacaatggg
 241 gaatggtgtg aagcccaaac caaaaatggc caaggctggg tcccaagcaa ctacatcacg
 301 ccagtcaaca gtctggagaa acactcctgg taccatgggc ctgtgtcccg caatgccgct
 361 gagtatctgc tgagcagcgg gatcaatggc agcttcttgg tgcgtgagag tgagagcagt
 421 cctggccaga ggtccatctc gctgagatac gaagggaggg tgtaccatta caggatcaac
 481 actgcttctg atggcaagct ctacgtctcc tccgagagcc gcttcaacac cctggccgag
 541 ttggttcatc atcattcaac ggtggccgac gggctcatca ccacgctcca ttatccagcc
 601 ccaaagcgca acaagcccac tgtctatggt gtgtccccca actacgacaa gtgggagatg
 661 gaacgcacgg acatcaccat gaagcacaag ctgggcgggg gccagtacgg ggaggtgtac
 721 gagggcgtgt ggaagaaata cagcctgacg gtggccgtga agaccttgaa ggaggacacc
 781 atggaggtgg aagagttctt gaaagaagct gcagtcatga aagagatcaa acaccctaac
 841 ctggtgcagc tccttggggt ctgcacccgg gagcccccgt tctatatcat cactgagttc
 901 atgacctacg ggaacctcct ggactacctg agggagtgca accggcagga ggtgaacgcc
 961 gtggtgctgc tgtacatggc cactcagatc tcgtcagcca tggagtacct ggagaagaaa
1021 aacttcatcc acagagatct tgctgcccga aactgcctgg taggggagaa ccacttggtg
1081 aaggtagctg attttggcct gagcaggttg atgacagggg acacctacac agcccctgct
1141 ggagccaagt tccccatcaa atggactgca cccgagagcc tggcctacaa caagttctcc
1201 atcaagtccg acgtctgggc atttggagta ttgctttggg aaattgctac ctatggcatg
```

Certificate of Analysis

```
1261 tccccttacc cgggaattga cctgtcccag gtgtatgagc tgctagagaa ggactaccgc
1321 atggagcgcc cagaaggctg cccagagaag gtctatgaac tcatgcgagc atgttggcag
1381 tggaatccct ctgaccggcc ctcctttgct gaaatccacc aagcctttga aacaatgttc
1441 caggaatcca gtatctcaga cgaagtggaa aaggagctgg ggaaacaagg cgtccgtggg
1501 gctgtgagta ccttgctgca ggccccagag ctgcccacca agacgaggac ctccaggaga
1561 gctgcagagc acagagacac cactgacgtg cctgagatgc ctcactccaa gggccaggga
1621 gagagcgatc ctctggacca tgagcctgcc gtgtctccat tgctccctcg aaaagagcga
1681 ggtcccccgg agggcggcct gaatgaagat gagcgccttc tccccaaaga caaaaagacc
1741 aacttgttca gcgccttgat caagaagaag aagaagacag ccccaacccc tcccaaacgc
1801 agcagctcct tccgggagat ggacggccag ccggagcgca gaggggccgg cgaggaagag
1861 ggccgagaca tcagcaacgg ggcactggct ttcacccccт tggacacagc tgacccagcc
1921 aagtccccaa agcccagcaa tggggctggg gtccccaatg gagccctccg ggagtccggg
1981 ggctcaggct tccggtctcc ccacctgtgg aagaagtcca gcacgctgac cagcagccgc
2041 ctagccaccg gcgaggagga gggcggtggc agctccagca agcgcttcct gcgctcttgc
2101 tccgcctcct gcgttcccca tggggccaag gacacggagt ggaggtcagt cacgctgcct
2161 cgggacttgc agtccacggg aagacagttt gactcgtcca catttggagg gcacaaaagt
2221 gagaagccgg ctctgcctcg gaagagggca ggggagaaca ggtctgacca ggtgacccga
2281 ggcacagtaa cgcctccccc caggctggtg aaaaagaatg aggaagctgc tgatgaggtc
2341 ttcaaagaca tcatggagtc cagcccgggc tccagcccgc ccaacctgac tccaaaaccc
2401 ctccggcggc aggtcaccgt ggcccctgcc tcgggcctcc cccacaagga agaagctgga
2461 aagggcagtg ccttagggac ccctgctgca gctgagccag tgacccccac cagcaaagca
2521 ggctcaggtg caccaggggg caccagcaag ggccccgccg aggagtccag agtgaggagg
2581 cacaagcact cctctgagtc gccagggagg gacaagggga aattgtccag gctcaaacct
2641 gccccgccgc ccccaccagc agcctctgca gggaaggctg gaggaaagcc ctcgcagagc
2701 ccgagccagg aggcggccgg ggaggcagtc ctgggcgcaa agacaaaagc cacgagtctg
2761 gttgatgctg tgaacagtga cgctgccaag cccagccagc cgggagaggg cctcaaaaag
2821 cccgtgctcc cggccactcc aaagccacag tccgccaagc cgtcggggac ccccatcagc
2881 ccagcccccg ttccctccac gttgccatca gcatcctcgg ccctggcagg ggaccagccg
2941 tcttccaccg ccttcatccc tctcatatca acccgagtgt ctcttcggaa aacccgccag
3001 cctccagagc ggatcgccag cggcgccatc accaaggggcg tggtcctgga cagcaccgag
3061 gcgctgtgcc tcgccatctc taggaactcc gagcagatgg ccagccacag cgcagtgctg
3121 gaggccggca aaaacctcta cacgttctgc gtgagctatg tggattccat ccagcaaatg
3181 aggaacaagt ttgccttccg agaggccatc aacaaactgg agaataatct ccgggagctt
3241 cagatctgcc cggcgacagc aggcagtggt ccagcggcca ctcaggactt cagcaagctc
3301 ctcagttcgg tgaaggaaat cagtgatata gtgcagaggt ag
```

Reviewed and approved by site quality representative.