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Certificate of Analysis

Abl (mouse), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-459, 14-459-K, 14-459M
Parent Lot # LB164P86

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 mouse Abl, amino acids 27–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 61% by SDS-PAGE and Coomassie blue staining. MW = 123.5kDa.

Specific Activity (Parent lot# LB164P86): 1195U/mg, where one unit of Abl (mouse), 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: 3.19mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1 % 2-mercaptoethanol. Frozen solution.

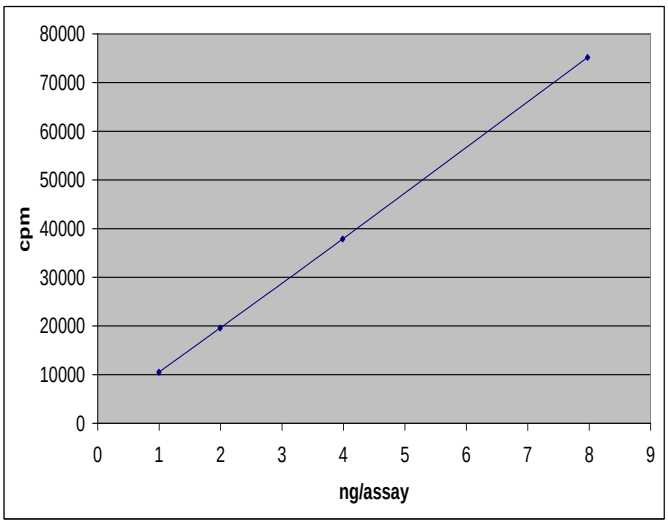
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

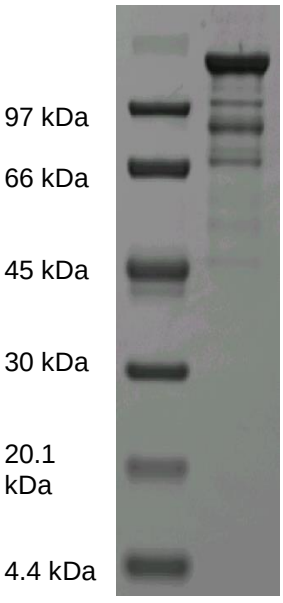
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1–8ng 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 Abl (mouse) with the translated sequence listed on pages three and four.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Abl (mouse), 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 (mouse), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–8ng 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 (EAIYAAPFAKKK)**.
3. Add **2.5µl (1–8ng) Abl (mouse), 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.

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Mouse Abl Sequence Information

Protein mouse Abl

Tags *N*-Terminal 6His

Native sequence E30 of the recombinant protein is equivalent to E27 of mouse Abl

Accession number GenBank J02995. The recombinant protein contains four amino acids which conflict with J02995, these are L782P, G784R, W785L, and L786V, and are reported in GenBank XM_130089.

Recombinant Mouse Abl amino acid sequence:

```
   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPGE ALQRPVASDF EPQGLSEAAR WNSKENLLAG
  61 PSENDPNLFV ALYDFVASGD NTLSITKGEK LRVLGYNHNG EWCEAQTKNG QGWVPSNYIT
 121 PVNSLEKHSW YHGPVSRNAA EYLLSSGING SFLVRESESS PGQRSISLRY EGRVYHYRIN
 181 TASDGKLYVS SESRFNTLAE LVHHHSTVAD GLITTLHYPA PKRNKPTIYG VSPNYDKWEM
 241 ERTDITMKHK LGGGQYGEVY EGVWKKYSLT VAVKTLKEDT MEVEEFLKEA AVMKEIKHPN
 301 LVQLLGVCTR EPPFYIITEF MTYGNLLDYL RECNRQEVSA VVLLYMATQI SSAMEYLEKK
 361 NFIHRDLAAR NCLVGENHLV KVADFGLSRL MTGDTYTAHA GAKFPIKWTA PESLAYNKFS
 421 IKSDVWAFGV LLWEIATYGM SPYPGIDLSQ VYELLEKDYR MERPEGCPEK VYELMRACWQ
 481 WNPSDRPSFA EIHQAFETMF QESSISDEVE KELGKRGTRG GAGSMLQAPE LPTKTRTCRR
 541 AAEQKDAPDT PELLHTKGLG ESDALDSEPA VSPLLPRKER GPPDGSLNED ERLLPRDRKT
 601 NLFSALIKKK KKMAPTPPKR SSSFREMDGQ PDRRGASEDD SRELCNGPPA LTSDAAEPTK
 661 SPKASNGAGV PNGAFREPGN SGFRSPHMWK KSSTLTGSRL AAAEEESGMS SSKRFLRSCS
 721 ASCMPHGARD TEWRSVTLPR DLPSAGKQFD SSTFGGHKSE KPALPRKRTS ESRSEQVAKS
 781 TAMPPPRLVK KNEEAAEEGF KDTESSPGSS PPSLTPKLLR RQVTASPSSG LSHKEEATKG
 841 SASGMGTPAT AEPAPPSNKV GLSKASSEEM RVRRHKHSSE SPGRDKGRLA KLKPAPPPPP
 901 ACTGKAGKPA QSPSQEAGEA GGPTKTKCTS LAMDAVNTDP TKAGPPGEGL RKPVPPSVPK
 961 PQSTAKPPGT PTSPVSTPST APAPSPLAGD QQPSSAAFIP LISTRVSLRK TRQPPERIAS
1021 GTITKGVVLD STEALCLAIS RNSEQMASHS AVLEAGKNLY TFCVSYVDSI QQMRNKFAFR
1081 EAINKLESNL RELQICPATA SSGPAATQDF SKLLSSVKEI SDIVRR
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Recombinant Mouse Abl nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccgggcgaa gccctgcaga ggccagtggc atctgacttt
 121 gagccccagg gtctcagcga agcagctcga tggaactcca aggaaaacct tcttgctggg
 181 cccagtgaaa atgaccccaa ccttttttgtg gcactctatg attttgtggc cagtggagat
 241 aacactctca gcatcactaa aggtgaaaag ctccgggtct tgggttataa tcacaatggg
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 361 cccgtcaaca gcctggagaa acattcctgg tatcatggcc ctgtatctcg gaatgctgct
 421 gagtatctgc tgagcagcgg aatcaacggc agcttcttag tgcgggagag tgagagtagc
 481 cctggccaga gatccatctc gctgcggtat gaagggaggg tgtaccacta caggatcaac
 541 actgcctctg atggcaagct gtacgtgtcc tccgagagcc gcttcaacac tctggctgag
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 661 cccaagcgca acaagcccac tatctacggt gtgtccccca actacgacaa gtgggaaatg
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Reviewed and approved by site quality representative.