

Certificate of Analysis

Abl, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-529, 14-529-K, 14-529M

Parent Lot # D9SN013N

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, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 79% by SDS-PAGE and Coomassie blue staining. MW = 121kDa.

Specific Activity (Parent lot# D9SN013N): 1067U/mg, where one unit of Abl activity is defined as 1nmol phosphate incorporated into 50µM Abltide (EAIYAAPFAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.852mg/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 6 months 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.

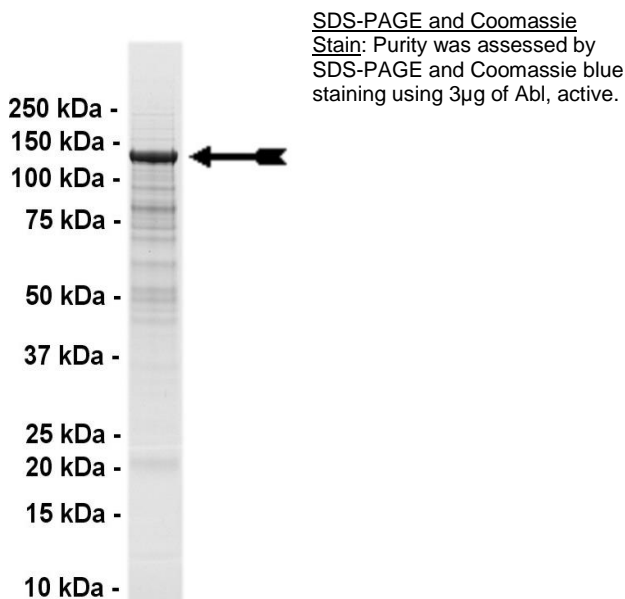
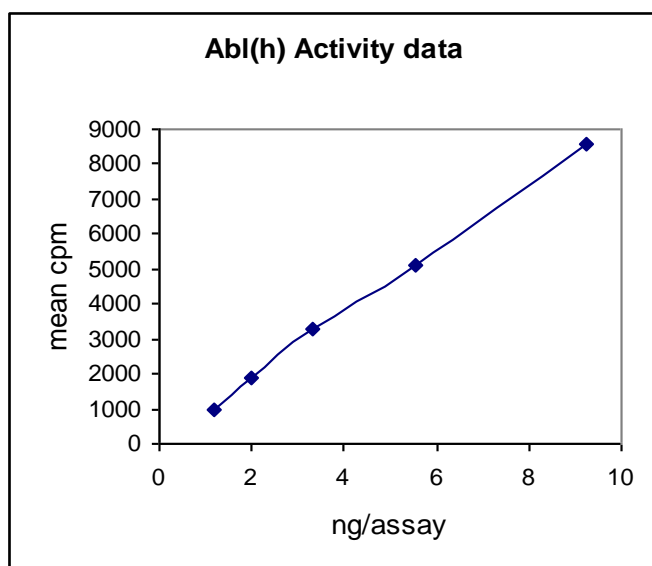
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 1.2–9.3ng 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 product identity as Abl 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 50 μ M. Prepare a 500 μ M stock and add 2.5 μ l of stock per assay point.
- 3. Abl, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.2–9.3ng 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 per assay to wells.
2. Add 2.5 μ l of **Abltide**.
3. Add **2.5 μ l (1.2–9.3ng) 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.

Certificate of Analysis

Abl Sequence Information

Protein	human Abl
Tags	N-terminal 6His
Native sequence	E10 of the recombinant protein is equivalent to E27 of human Abl
Accession number	GenBank U07563

Recombinant Abl amino acid sequence:

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1 MHHHHHHEFE ALQRPVASDF EPQGLSEAR WNSKENLLAG PSENDPNLFV ALYDFVASGD
61 NTLISITKGEK LRVLGYNHNG EWCEAQTNG 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 MTGDITYTAHA GAKFPIKWTA PESLAYNKFS IKSDVWAFGV LLWEIATYGM
421 SPYPGIDLSQ VYELLEKDYR MERPEGCPEK VYELMRACWQ WNPDRPSFA EIHQAFETMF
481 QESSISDEVE KELGKQGVRG AVSTLLQAPE LPTKTRTSRR AAEHRDSTDV PEMPHSKGQG
541 ESDPLDHEPA VSPLLPRKER GPPEGGLNED ERLLPKDKKT NLFSALIKKK KKTAPTPPKR
601 SSSFREMDGQ PERRGAGEEE GRDISNGALA FTPLDTADPA KSPKPSNGAG VPNGALRESG
661 GSGFRSPHLW KKSSTLTSSR LATGEEEGGG SSSKRFLRSC SASCVPHGAK DTEWRSVTLF
721 RDLQSTGRQF DSSTFGGHKS EKPALPRKRA GENRSDQVTR GTVTPPPRLV KKNEEADEV
781 FKDIMESSPG SSPPNLTPKP LRRQVTVAPA SGLPHKEEAG KGSALGTPAA AEPVTPTSKA
841 GSGAPGGTSK GPAEESRVRH HKHSSESPPR DKGKLSRLKP APPPPAASA GKAGGKPSQS
901 PSQEAAGEAV LGAKTKATSL VDAVNSDAK PSQPGGLKK PVLPATPKPQ SAKPSGTPIS
961 PAPVPSTLPS ASSALAGDQP SSTAFIPLIS TRVSLRKTRO PPERIASGAI TKGVVLDSTE
1021 ALCLAISRNS EQMASHSAVL EAGKNLYTFC VSYVDSIQQM RNKFVAFREAI NKLENNLREL
1081 QICPATAGSG PAATQDFSKL LSSVKEISDI VQR
    
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Recombinant Abl nucleotide sequence:

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1 atgcatcatc accatcacca tgaattcgaa gcccttcagc ggccagtagc atctgacttt
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301 ccagtcaaca gtctggagaa aactcctgg taccatgggc ctgtgtcccg caatgccgct
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481 actgcttctg atggcaagct ctacgtctcc tccgagagcc gcttcaacac cttggccgag
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Certificate of Analysis

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