

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ABL1 [118 - 535]

<u>Enzyme description:-</u>	ABL1 [118 - 535]
<u>Clone number:-</u>	DU 5578
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i> , co-expressed with His-YopH [DU 31259]
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose
<u>Calculated molecular mass:-</u>	
Monoisotopic	74, 424.49 daltons
Average Mass	74, 472.32 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.00
<u>Purity:-</u>	>80 %
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF	
<u>Storage temperature:-</u>	-70 °C
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
ABLtide [EAIYAAPFAKKK]	Final concentration: 300 µM

Division of Signal Transduction Therapy

Clone Data Sheet

ABL1 [118 - 535]

Protein ABL1 [118 - 535]

Clone number DU 5578

Species Human

Accession number NM_005157

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGPLGSPVNSLEKHS
WYHGPVSRNAEYLLSSGINGSFLVRESESSPGQRSISLRYEGRVYHY
RINTASDGKLYVSSESFRNTLAELVHHHSTVADGLITTLHYPAPKRNK
PTVYGVSPNYDKWEMERTDITMKHKLGGGQYGEVYEGVWKKYSLTVAV
KTLKEDTMEVEEFLKEAAVMKEIKHPNLVQLLGVCRTREPPFYIITEFM
TYGNLLDYLRECNRQEVNAVLLYMATQISSAMEYLEKKNFIHRDLAA
RNCLVGENHLVKVADFGLSRLMTGDTYTAHAGAKFPIKWTAPESLAYN
KFSIKSDVWAFGVLLWEIATYGMSPYPGIDLSQVYELLEKDYRMERPE
GCPEKVYELMRACWQWNPSDRPSFAEIHQAFETMFQESSISDEVEKEL
GKQGVRGAVSTLLQAPELPTKTRTS

Native sequence Amino acids P118 – S535 of human ABL1.
[Full length protein ends at residue R1130]

Residue P232 of the fusion protein is equivalent to P2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage Prescission site (LEVLFQGP) at residues 221 - 228

Cloning sites *Bam*H1 and *Eco*R1 sites of pGEX-6P-1

Division of Signal Transduction Therapy

Nucleotide sequence of insert

ggatcccCAGTCAACAGTCTGGAGAAACACTCCTGGTACCATGGGCCT
GTGTCCCGCAATGCCGCTGAGTATCTGCTGAGCAGCGGGATCAATGGC
AGCTTCTTGGTGCGTGAGAGTGAGAGCAGTCCTGGCCAGAGGTCCATC
TCGCTGAGATACGAAGGGAGGGTGTACCATTACAGGATCAACACTGCT
TCTGATGGCAAGCTCTACGTCTCCTCCGAGAGCCGCTTCAACACCCTG
GCCGAGTTGGTTCATCATCATTCAACGGTGGCCGACGGGCTCATCACC
ACGCTCCATTATCCAGCCCCAAAGCGCAACAAGCCCACCTGTCTATGGT
GTGTCCCCCAACTACGACAAGTGGGAGATGGAACGCACGGACATCACC
ATGAAGCACAAGCTGGGCGGGGGCCAGTACGGGGAGGTGTACGAGGGC
GTGTGGAAGAAATACAGCCTGACGGTGGCCGTGAAGACCTTGAAGGAG
GACACCATGGAGGTGGAAGAGTTCTTGAAAGAAGCTGCAGTCATGAAA
GAGATCAAACACCCTAACCTGGTGCAGCTCCTTGGGGTCTGCACCCGG
GAGCCCCCGTTCTATATCATCACTGAGTTCATGACCTACGGGAACCTC
CTGGACTACCTGAGGGAGTGCAACCGGCAGGAGGTGAACGCCGTGGTG
CTGCTGTACATGGCCACTCAGATCTCGTCAGCCATGGAGTACCTGGAG
AAGAAAACTTCATCCACAGAGATCTTGCTGCCCCGAAACTGCCTGGTA
GGGAGAACTTGGTGAAGGTAGCTGATTTTGGCCTGAGCAGGTTG
ATGACAGGGGACACCTACACAGCCCATGCTGGAGCCAAGTTCCCCATC
AAATGGACTGCACCCGAGAGCCTGGCCTACAACAAGTTCTCCATCAAG
TCCGACGTCTGGGCATTTGGAGTATTGCTTTGGGAAATTGCTACCTAT
GGCATGTCCCCTTACCCGGAATTGACCTGTCCCAGGTGTATGAGCTG
CTAGAGAAGGACTACCGCATGGAGCGCCCAGAAGGCTGCCCAGAGAAG
GTCTATGAACTCATGCGAGCATGTTGGCAGTGGAATCCCTCTGACCGG
CCCTCCTTTGCTGAAATCCACCAAGCCTTTGAAACAATGTTCCAGGAA
TCCAGTATCTCAGACGAAGTGGAAAAGGAGCTGGGGAAACAAGGCGTC
CGTGGGGCTGTGAGTACCTTGCTGCAGGCCCCAGAGCTGCCCACCAAG
ACGAGGACCTCCtaggaattc