Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ABL1 [118 - 535]

Enzyme description:- ABL1 [118 - 535]

Clone number:- DU 5578

Source:- Recombinant

Expression system:- *E.coli*, co-expressed with His-YopH [DU 31259]

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 74, 424.49 daltons
Average Mass 74, 472.32 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.00

Purity:- >80 %

Enzyme storage buffer:-
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

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
ABLtide [EAIYAAPFAKKK] Final concentration: 300 μM

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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 GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**PVNSLEKHS** **WYHGPVSRNAAEYLLSSGINGSFLVRESESSPGQRSISLRYEGRVYHY** **RINTASDGKLYVSSESRFNTLAELVHHHSTVADGLITTLHYPAPKRNK** **PTVYGVSPNYDKWEMERTDITMKHKLGGGQYGEVYEGVWKKYSLTVAV** **KTLKEDTMEVEEFLKEAAVMKEIKHPNLVQLLGVCTREPPFYIITEFM** **TYGNLLDYLRECNRQEVNAVVLLYMATQISSAMEYLEKKNFIHRDLAA** **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

Nucleotide sequence of insert

ggatccCCAGTCAACAGTCTGGAGAAACACTCCTGGTACCATGGGCCT
GTGTCCCGCAATGCCGCTGAGTATCTGCTGAGCAGCGGGATCAATGGC
AGCTTCTTGGTGCGTGAGAGTGAGAGCAGTCCTGGCCAGAGGTCCATC
TCGCTGAGATACGAAGGGAGGGTGTACCATTACAGGATCAACACTGCT
TCTGATGGCAAGCTCTACGTCTCCTCCGAGAGCCGCTTCAACACCCTG
GCCGAGTTGGTTCATCATCATTCAACGGTGGCCGACGGGCTCATCACC
ACGCTCCATTATCCAGCCCCAAAGCGCAACAAGCCCACTGTCTATGGT
GTGTCCCCCAACTACGACAAGTGGGAGATGGAACGCACGGACATCACC
ATGAAGCACAAGCTGGGCGGGGGCCAGTACGGGGAGGTGTACGAGGGC
GTGTGGAAGAAATACAGCCTGACGGTGGCCGTGAAGACCTTGAAGGAG
GACACCATGGAGGTGGAAGAGTTCTTGAAAGAAGCTGCAGTCATGAAA
GAGATCAAACACCCTAACCTGGTGCAGCTCCTTGGGGTCTGCACCCGG
GAGCCCCCGTTCTATATCATCACTGAGTTCATGACCTACGGGAACCTC
CTGGACTACCTGAGGGAGTGCAACCGGCAGGAGGTGAACGCCGTGGTG
CTGCTGTACATGGCCACTCAGATCTCGTCAGCCATGGAGTACCTGGAG
AAGAAAAACTTCATCCACAGAGATCTTGCTGCCCGAAACTGCCTGGTA
GGGGAGAACCACTTGGTGAAGGTAGCTGATTTTGGCCTGAGCAGGTTG
ATGACAGGGGACACCTACACAGCCCATGCTGGAGCCAAGTTCCCCATC
AAATGGACTGCACCCGAGAGCCTGGCCTACAACAAGTTCTCCATCAAG
TCCGACGTCTGGGCATTTGGAGTATTGCTTTGGGAAATTGCTACCTAT
GGCATGTCCCCTTACCCGGGAATTGACCTGTCCCAGGTGTATGAGCTG
CTAGAGAAGGACTACCGCATGGAGCGCCCAGAAGGCTGCCCAGAGAAG
GTCTATGAACTCATGCGAGCATGTTGGCAGTGGAATCCCTCTGACCGG
CCCTCCTTTGCTGAAATCCACCAAGCCTTTGAAACAATGTTCCAGGAA
TCCAGTATCTCAGACGAAGTGGAAAAGGAGCTGGGGAAACAAGGCGTC
CGTGGGGCTGTGAGTACCTTGCTGCAGGCCCCAGAGCTGCCCACCAAG
ACGAGGACCTCCtaggaattc