

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of DEBRIN1 [1 - 649]

Enzyme description:- DEBRIN1 [1 - 649]

Clone number:- DU 38606

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 98, 188.08 daltons

Average Mass 98, 249.47 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.57

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

Debrin1 [1 – 649]

Protein Debrin1 [1 – 649]

Clone number DU 38606

Species Human

Accession number AAA16256.1

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMAGVSFSGHRLELL
AAEVEVIREESAADWALYTYEDGSDDLKLAASGEGGLQELSGHFENQKV
MYGFCSVKDSQAALPKYVLINWVGEDVDPDARKCACASHVAKVAEFFQGV
DVI VNASSVEDIDAGAI GQRLSNGLARLSSPVLHRLRLREDENAEPVGT
TYQKTDAAVEMKRINREQFWEQAKKEEELRKEEERKKALDERLRFQER
MEQERQEQEERERRREREREQOIEEHRRKQOTLEAEEAKRRLKEQSIFGD
HRDEEEETHMKKSESEVEEAAA IIAQRPDNPREFFKQQERVASASAGSC
DVPSPFNHRPGSHLDSHRRMAPTPIPTRSPSDSSTASTPVAEQIERALD
EVTSSQPPPLPPPPPPAQETQEPSPILDSEETRAAAPQAWAGPMEEPPQ
AQAPPRGPGSPAEDLMFESAEQAVLAAPVEPATADATEVHDAADTIET
DTATADTTVANNVPPAATSLIDLWPGNGEGASTLQGEPRAPTPPSGTEV
TLAEVPLLDEVAPELLPAGEGCATLLNFDELPEPPATFCDPEEVEGEP
LAAPQTPTLPSALEELEQEQEPEPHLLTNGETTQKEGTQASEGYFSQSQ
EEEFAQSEELCAKAPPPVFYNKPPEIDITCWDADVPVEEEEGFEGGD

Native sequence Amino acids M1 – D649 (end) of human Debrin1.
Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*1 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccATGGCCGGCGTCAGCTTCAGCGGCCACCGCCTGGAGCTGCTGGC
GGCTTACGAGGAGGTGATCCGAGAGGAGAGCGCGGCCACTGGGCTCTGT
ACACATATGAAGATGGCTCCGATGACCTCAAGCTTGCAGCATCAGGAGAA
GGGGGCTTGCAGGAGCTTTCGGGACACTTTGAGAACCAGAAGGTGATGTA
CGGCTTCTGCAGTGTCAAGGACTCCCAAGCTGCTCTGCCAAAATACGTGC
TCATCAACTGGGTGGGCGAAGATGTGCCTGATGCCCCGAAGTGCGCTTGT
GCCAGCCACGTGGCTAAGGTGGCAGAGTTCTTCCAGGGTGTGCACGTGAT
CGTGAACGCCAGCAGCGTGGAAGACATAGACGCGGGGTGCCATCGGGCAGC
GGCTCTCTAACGGGCTGGCGCGACTCTCCAGCCCTGTGCTGCACCGACTG
CGGCTGCGAGAGGATGAGAACGCAGAGCCCCTGGGCACCACCTACCAGAA
GACGGATGCAGCTGTGGAATGAAGCGGATTAACCGAGAGCAGTTCTGGG
AGCAGGCCAAGAAGGAAGAAGAGCTGCGGAAGGAGGAGGAGCGGAAGAAG
GCCCTGGATGAGAGGCTCAGGTTTCGAGCAGGAGCGGATGGAGCAGGAGCG
GCAGGAGCAAGAGGAGCGCGAGCGGCGCTACCGGGAGCGGGAGCAGCAGA
TCGAGGAGCACAGGAGGAAACAGCAGACTTTAGAAGCGGAAGAGGCCAAG
AGGCGGTTGAAGGAGCAGTCTATCTTTGGTGACCATCGGGATGAGGAGGA
AGAGACCCACATGAAGAAATCAGAGTCCGAGGTTGGAGGAGGCAGCAGCTA
TTATTGCCCAGCGGCCTGACAACCCAAGGGAGTTCTTCAAGCAGCAGGAA
AGAGTCGCATCGGCCTCTGCGGGCAGCTGTGATGTACCCTCGCCCTTCAA
CCATCGACCAGGCAGCCACCTGGACAGCCACCGGAGGATGGCGCCCACTC
CCATCCCCACGCGGAGCCCCTGACTCCAGCACCGCCTCCACCCCTGTC
GCTGAGCAGATAGAGCGGGCCCTGGATGAGGTCACCTCCTCGCAGCCTCC
ACCACTGCCACCGCCACCCCCACCAGCCCAAGAGACCCAGGAGCCAGCC
CCATCCTAGACAGTGAGGAGACCAGAGCAGCAGCCCCCTCAGGCCTGGGCC
GGCCCCATGGAGGAGCCCCCTCAGGCACAGGCGCCTCCCCGGGGGCCAGG
CAGCCCTGCAGAGGACTTGATGTTTCATGGAGTCTGCAGAGCAGGCTGTCC
TGGCTGCTCCCGTGGAGCCTGCCACAGCTGACGCCACGGAGGTCCACGAT
GCAGCTGACACCATTGAAACTGACACTGCCACTGCTGACACCACTGTTGC
CAACAACGTACCCCCCGCCGCCACCAGCCTCATTTGACCTATGGCCTGGCA
ACGGGGAAGGGGCCTCCACACTCCAGGGTGAGCCAGGGCCCCCACGCCA
CCCTCGGGTACTGAGGTCACCCTGGCAGAGGTGCCCTGCTGGATGAGGT
GGCTCCGGAGCCACTGCTGCCAGCAGGCGAAGGCTGTGCCACCCTTCTCA
ACTTTGATGAGCTGCCTGAGCCGCCAGCCACCTTCTGTGACCCAGAGGAA
GTGGAAGGGGAGCCCCTGGCTGCCCCCCAGACCCCAACTCTGCCCTCAGC
CCTTGAGGAGCTGGAGCAAGAGCAGGAGCCGGAGCCCCACCTGCTAACCA
ATGGCGAGACCACCAGAAGGAGGGGACCCAGGCCAGTGAGGGGTACTTC
AGTCAATCACAGGAGGAGGAGTTTGCCCAATCGGAAGAGCTCTGTGCCAA
GGCTCCGCCTCCTGTGTTCTACAACAAGCCTCCAGAGATCGACATCACAT
GCTGGGATGCAGACCCAGTTCCAGAAGAGGAGGAGGGCTTCGAGGGTGGT
GAttagcggccgc