

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of ABIN1 D472N L490P [1 - 636]

Enzyme description:- ABIN1 D472N L490P [1 – 636]

Clone number:- DU 8697

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 98, 635.22 daltons

Average Mass 98, 697.40 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.13

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

ABIN1 D472N L490P [1 – 636]

<u>Protein</u>	ABIN1 D472N L490P [1 – 636]
<u>Clone number</u>	DU 8697
<u>Species</u>	Human
<u>Accession number</u>	NM_006058.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLRYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVLV FQGPLGSMEGRGPYRIYDPGG SVPSGEASAAFERLVKENSRLKEKMQGIKMLGELLEESQMEATRLRQKA EELVKDNELLPPSPSLGSDPLAELTGKDSNVTASPTAPACPSDKPAP VQKPPSSGTSSEFEVVTPEEQNSPESSSHANAMALGPLPREDGNLMLHL QRLETTLSVCAEEP DHGQLFTHLGRMALEFNRLASKVHKNEQRTSILQT LCEQLRKENEALKAKLDKGLEQRDQAAERLREENLELKKLLMSNGNKEG ASGRPGSPKMEGTGKKAVAGQQQASVTAGKVPEVVALGAPEKKVKMLEQ QRSELLEVNKQWDQHFRSMKQOYEQKITELRQKLADLQKQVTDLEAERE QKQRDFDRKLLLA KSKIEMEETDKEQLTAEAKELRQKVYLODQLSPLT RQREYQEKIQR LNKALEEALS IQTPPSSPPTAFGSPEGAGALLRKQEL VTQNE LLKQOVKIF EENFQRERSDRERMNEEK EEPKKQVEKLQAQVTL S NAQLKAFKDEEKAREALRQQRKAKASGERYHVEPHPEHL CGAYPYAYP PMPAMVPHHG FEDWSQIRYPPPPMAMEHPPPLPNSRLFHLPEYTWRLPC GGVRNPNQSSQVMDPPTARPTEPESPKNDR EG PQ</p>
<u>Native sequence</u>	<p>Amino acids M1 – Q636 (end) of human ABIN1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220. The protein has a D472N and a L490P mutation. Residue D472 is equivalent to residue N703 of the fusion protein and residue L490 is equivalent to residue P721 of the fusion protein.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccATGGAAGGGAGAGGACCGTACCGGATCTACGACCCTGGGGGCA
GCGTGCCCTCAGGAGAGGCATCCGCAGCTTTTGAGCGCCTAGTGAAGGA
GAATTCCCGGCTGAAGGAAAAAATGCAAGGGATAAAGATGTTAGGGGAG
CTTTTGGAAAGAGTCCCAGATGGAAGCGACCAGGCTCCGGCAGAAGGCAG
AGGAGCTAGTGAAGGACAACGAGCTGCTCCCACCACCTTCTCCCTCCTT
GGGCTCCTTCGACCCCTGGCTGAGCTCACAGGAAAGGACTCAAATGTC
ACAGCATCTCCCACAGCCCCTGCATGCCCCAGTGACAAGCCAGCACCAG
TCCAGAAGCCTCCATCCAGTGGCACCTCCTCTGAATTTGAAGTGGTCAC
TCCTGAGGAGCAGAATTCACCAGAGAGCAGCAGCCATGCCAATGCGATG
GCGCTGGGCCCCCTGCCCGTGAGGACGGCAACCTGATGCTGCACCTGC
AGCGCCTGGAGACCACGCTGAGTGTGTGTGCCGAGGAGCCGGACCACGG
CCAGCTCTTACCCACCTGGGCCGCATGGCCCTGGAGTTCAACCGACTG
GCATCCAAGGTGCACAAGAATGAGCAGCGCACCTCCATTCTGCAGACCC
TGTGTGAGCAGCTTCGGAAGGAGAACGAGGCTCTGAAGGCCAAGTTGGA
TAAGGGCCTGGAACAGCGGGATCAGGCTGCCGAGAGGCTGCGGGAGGAA
AATTTGGAGCTCAAGAAGTTGTTGATGAGCAATGGCAACAAAGAGGGTG
CGTCTGGGCGGCCAGGCTCACCGAAGATGGAAGGGACAGGCAAGAAGGC
AGTGGCTGGACAGCAGCAGGCTAGTGTGACGGCAGGTAAGGTCCCAGAG
GTGGTGGCCTTGGGCGCACCCGAGAAGAAGGTGAAGATGCTGGAGCAGC
AGCGCAGTGAGCTGCTGGAAGTGAACAAGCAGTGGGACCAGCATTTCCG
GTCCATGAAGCAGCAGTATGAGCAGAAGATCACTGAGCTGCGTCAGAAG
CTGGCTGATTTGCAGAAGCAGGTGACTGACCTGGAGGCCGAGCGGGAGC
AGAAGCAGCGTGACTTTGACCGCAAGCTCCTCCTGGCCAAGTCCAAGAT
TGAAATGGAGGAGACCGACAAGGAGCAGCTGACAGCAGAGGCCAAGGAG
CTGCGCCAAAAGGTCAAGTACCTGCAGGATCAGCTGAGCCCCTCACCC
GACAGCGTGAGTACCAGGAAAAGGAGATCCAGCGGCTCAACAAGGCCCT
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TCACGCAGAATGAGTTGCTGAAACAGCAGGTGAAGATCTTCGAGGAGAA
CTTCCAGAGGGAGCGCAGTGATCGTGAGCGCATGAATGAGGAGAAGGAA
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CAGACAGCAGAAGAGGAAAAGCAAAGGCCCTCAGGAGAGCGTTACCATGTG
GAGCCCCACCCAGAACATCTCTGCGGGCCTACCCCTACGCCTACCCGC
CCATGCCAGCCATGGTGCCACACCATGGCTTCGAGGACTGGTCCCAGAT
CCGCTACCCCTCCCCCATGGCCATGGAGCACCCGCCCCCACTCCCC
AACTCGCGCCTCTTCCATCTGCCGGAATACACCTGGCGTCTACCTTGTG
GAGGGGTTCGAAATCCAAATCAGAGCTCCCAAGTGATGGACCCTCCCAC
AGCCAGGCCTACAGAACCAGAGTCTCCAAAAAATGACCGTGAGGGGCCT
CAGtgagcggccgc