Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of ABIN1 D472N [1 - 636]

Enzyme description:-	ABIN1 D472N [1 – 636]
Clone number:-	DU 8695
Source:-	Recombinant
Expression system:-	*E.coli*
Tag:-	N-terminal GST
Purification method:-	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	98, 651.25 daltons
Average Mass	98, 713.45 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

Clone Data Sheet

ABIN1 D472N [1 – 636]

Protein	ABIN1 D472N [1 – 636]
Clone number	DU 8695
Species	Human
Accession number	NM_006058.4
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MEGRGPYRIYDPGG** **SVPSGEASAAFERLVKENSRLKEKMQGIKMLGELLEESQMEATRLRQKA** **EELVKDNELLPPPSPSLGSFDPLAELTGKDSNVTASPTAPACPSDKPAP** **VQKPPSSGTSSEFEVVTPEEQNSPESSSHANAMALGPLPREDGNLMLHL** **QRLETTLSVCAEEPDHGQLFTHLGRMALEFNRLASKVHKNEQRTSILQT** **LCEQLRKENEALKAKLDKGLEQRDQAAERLREENLELKKLLMSNGNKEG** **ASGRPGSPKMEGTGKKAVAGQQQASVTAGKVPEVVALGAPEKKVKMLEQ** **QRSELLEVNKQWDQHFRSMKQQYEQKITELRQKLADLQKQVTDLEAERE** **QKQRDFDRKLLLAKSKIEMEETDKEQLTAEAKELRQKVKYLQDQLSPLT** **RQREYQEKEIQRLNKALEEALSIQTPPSSPPTAFGSPEGAGALLRKQEL** **VTQNELLKQQVKIFEENFQRERSDRERMNEEKEELKKQVEKLQAQVTLS** **NAQLKAFKDEEKAREALRQQKRKAKASGERYHVEPHPEHLCGAYPYAYP** **PMPAMVPHHGFEDWSQIRYPPPPMAMEHPPPLPNSRLFHLPEYTWRLPC** **GGVRNPNQSSQVMDPPTARPTEPESPKNDREGPQ**
Native sequence	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 mutation. Residue D472 is equivalent to residue N703 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

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ggatccATGGAAGGGAGAGGACCGTACCGGATCTACGACCCTGGGGGCA
GCGTGCCCTCAGGAGAGGCATCCGCAGCTTTTGAGCGCCTAGTGAAGGA
GAATTCCCGGCTGAAGGAAAAAATGCAAGGGATAAAGATGTTAGGGGAG
CTTTTGGAAGAGTCCCAGATGGAAGCGACCAGGCTCCGGCAGAAGGCAG
AGGAGCTAGTGAAGGACAACGAGCTGCTCCCACCACCTTCTCCCTCCTT
GGGCTCCTTCGACCCCCTGGCTGAGCTCACAGGAAAGGACTCAAATGTC
ACAGCATCTCCCACAGCCCCTGCATGCCCCAGTGACAAGCCAGCACCAG
TCCAGAAGCCTCCATCCAGTGGCACCTCCTCTGAATTTGAAGTGGTCAC
TCCTGAGGAGCAGAATTCACCAGAGAGCAGCAGCCATGCCAATGCGATG
GCGCTGGGCCCCCTGCCCCGTGAGGACGGCAACCTGATGCTGCACCTGC
AGCGCCTGGAGACCACGCTGAGTGTGTGTGCCGAGGAGCCGGACCACGG
CCAGCTCTTCACCCACCTGGGCCGCATGGCCCTGGAGTTCAACCGACTG
GCATCCAAGGTGCACAAGAATGAGCAGCGCACCTCCATTCTGCAGACCC
TGTGTGAGCAGCTTCGGAAGGAGAACGAGGCTCTGAAGGCCAAGTTGGA
TAAGGGCCTGGAACAGCGGGATCAGGCTGCCGAGAGGCTGCGGGAGGAA
AATTTGGAGCTCAAGAAGTTGTTGATGAGCAATGGCAACAAAGAGGGTG
CGTCTGGGCGGCCAGGCTCACCGAAGATGGAAGGGACAGGCAAGAAGGC
AGTGGCTGGACAGCAGCAGGCTAGTGTGACGGCAGGTAAGGTCCCAGAG
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AGCCAGGCCTACAGAACCAGAGTCTCCAAAAAATGACCGTGAGGGGCCT
CAGtgagcggccgc
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