Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of ABIN1 L490P [1 - 636]

Enzyme description:- ABIN1 L490P [1 – 636]

Clone number:- DU 8696

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 98, 636.21 daltons
Average Mass 98, 698.39 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.07

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 L490P [1 – 636]

Protein	ABIN1 L490P [1 – 636]
Clone number	DU 8696
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** **VTQNELLKQQVKIFEEDFQRERSDRERMNEEKEE**P**KKQVEKLQAQVTLS** **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 L490P mutation. Residue L490 is equivalent to residue P721 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

ggatccATGGAAGGGAGAGGACCGTACCGGATCTACGACCCTGGGGGCA
GCGTGCCCTCAGGAGAGGCATCCGCAGCTTTTGAGCGCCTAGTGAAGGA
GAATTCCCGGCTGAAGGAAAAAATGCAAGGGATAAAGATGTTAGGGGAG
CTTTTGGAAGAGTCCCAGATGGAAGCGACCAGGCTCCGGCAGAAGGCAG
AGGAGCTAGTGAAGGACAACGAGCTGCTCCCACCACCTTCTCCCTCCTT
GGGCTCCTTCGACCCCCTGGCTGAGCTCACAGGAAAGGACTCAAATGTC
ACAGCATCTCCCACAGCCCCTGCATGCCCCAGTGACAAGCCAGCACCAG
TCCAGAAGCCTCCATCCAGTGGCACCTCCTCTGAATTTGAAGTGGTCAC
TCCTGAGGAGCAGAATTCACCAGAGAGCAGCAGCCATGCCAATGCGATG
GCGCTGGGCCCCCTGCCCCGTGAGGACGGCAACCTGATGCTGCACCTGC
AGCGCCTGGAGACCACGCTGAGTGTGTGTGCCGAGGAGCCGGACCACGG
CCAGCTCTTCACCCACCTGGGCCGCATGGCCCTGGAGTTCAACCGACTG
GCATCCAAGGTGCACAAGAATGAGCAGCGCACCTCCATTCTGCAGACCC
TGTGTGAGCAGCTTCGGAAGGAGAACGAGGCTCTGAAGGCCAAGTTGGA
TAAGGGCCTGGAACAGCGGGATCAGGCTGCCGAGAGGCTGCGGGAGGAA
AATTTGGAGCTCAAGAAGTTGTTGATGAGCAATGGCAACAAAGAGGGTG
CGTCTGGGCGGCCAGGCTCACCGAAGATGGAAGGGACAGGCAAGAAGGC
AGTGGCTGGACAGCAGCAGGCTAGTGTGACGGCAGGTAAGGTCCCAGAG
GTGGTGGCCTTGGGCGCACCCGAGAAGAAGGTGAAGATGCTGGAGCAGC
AGCGCAGTGAGCTGCTGGAAGTGAACAAGCAGTGGGACCAGCATTTCCG
GTCCATGAAGCAGCAGTATGAGCAGAAGATCACTGAGCTGCGTCAGAAG
CTGGCTGATTTGCAGAAGCAGGTGACTGACCTGGAGGCCGAGCGGGAGC
AGAAGCAGCGTGACTTTGACCGCAAGCTCCTCCTGGCCAAGTCCAAGAT
TGAAATGGAGGAGACCGACAAGGAGCAGCTGACAGCAGAGGCCAAGGAG
CTGCGCCAAAAGGTCAAGTACCTGCAGGATCAGCTGAGCCCACTCACCC
GACAGCGTGAGTACCAGGAAAAGGAGATCCAGCGGCTCAACAAGGCCCT
GGAGGAAGCACTGAGCATCCAAACCCCGCCATCATCTCCACCAACAGCA
TTTGGGAGCCCAGAAGGAGCAGGGGCCCTCCTAAGGAAACAGGAGCTGG
TCACGCAGAATGAGTTGCTGAAACAGCAGGTGAAGATCTTCGAGGAGGA
CTTCCAGAGGGAGCGCAGTGATCGTGAGCGCATGAATGAGGAGAAGGAA
GAGCCGAAGAAGCAAGTGGAGAAGCTGCAGGCCCAGGTCACCCTGTCAA
ATGCCCAGCTAAAAGCATTCAAAGATGAGGAGAAGGCAAGAGAAGCCCT
CAGACAGCAGAAGAGGAAAGCAAAGGCCTCAGGAGAGCGTTACCATGTG
GAGCCCCACCCAGAACATCTCTGCGGGGCCTACCCCTACGCCTACCCGC
CCATGCCAGCCATGGTGCCACACCATGGCTTCGAGGACTGGTCCCAGAT
CCGCTACCCCCCTCCCCCCATGGCCATGGAGCACCCGCCCCCACTCCCC
AACTCGCGCCTCTTCCATCTGCCGGAATACACCTGGCGTCTACCTTGTG
GAGGGGTTCGAAATCCAAATCAGAGCTCCCAAGTGATGGACCCTCCCAC
AGCCAGGCCTACAGAACCAGAGTCTCCAAAAAATGACCGTGAGGGGCCT
CAGtgagcggccgc