

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of ABIN2 L327P [1 - 429]

Enzyme description:- ABIN1 L327P [1 – 429]

Clone number:- DU 8639

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 75, 488.10 daltons

Average Mass 75, 535.74 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.94

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

ABIN2 L327P [1 – 429]

<u>Protein</u>	ABIN2 L327P [1 – 429]
<u>Clone number</u>	DU 8639
<u>Species</u>	Human
<u>Accession number</u>	NM_024309.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMSRDPGSGGWEEAP RAAAALCTLYHEAGQRLRRLQDQLAARDAL IARLRARLAALEGDAAPSL VDALLEQVARFREQLRRQEGGAAEAQMRQEI ERLTERLEEKEREMOQLL SQPQHEREKEVVLLRRSMAEGERARAASDVLCSLANETHQLRRTL TAT AHMCQHAKCLDERQHAQRNVGERSPDQSEHTDGHTSVQSVIEKLQEN RLLKQKVTHVEDLNAKWQRYNASRDEYVRGLHAQLRGLQIPHEPELMRK EISRLNRQLEEKINDCAEVKQELAASRTARDAALERVQMLEQQILAYKD DFMSERADRERAQSRIQEP EEKVASLLHQVSWRQDSREPDAGRIHAGSK TAKYLAADALELMVPGGWRPGTGSQQPEPPAEGGHPGAVQRGQGDLOCP HCLQCFSDQGEELLRHVAECCQ</p>
<u>Native sequence</u>	<p>Amino acids M1 – Q429 (end) of human ABIN2. 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 L327P mutation. Residue L327 is equivalent to P558 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

ggatccATGTCCCGGGACCCGGGGTCTGGGCGGCTGGGAGGAGGCCCCGC
GCGCAGCTGCCGCGCTCTGCACCCTGTACCACGAGGCCGGACAGCGGCT
GCGCCGCCTGCAGGACCAGCTCGCTGCCCGCGACGCCCTCATCGCTCGC
CTCCGCGCCCGCCTGGCCGCGCTGGAGGGGGACGCCGCGCCGTCCCTAG
TGGACGCGCTGCTGGAGCAGGTTGCGCGCTTCCGGGAGCAGCTGCCAAG
GCAGGAGGGCGGCGCCGCCGAGGCCAGATGCGCCAGGAAATTGAGAGG
CTGACTGAGCGACTAGAAGAAAAAGAGAGGGAGATGCAGCAGCTGCTGA
GCCAGCCCCAACACGAGCGAGAGAAGGAAGTCGTCTTGTACGGAGGAG
CATGGCAGAAGGGGAGCGCGCCCGGGCCGCCAGTGACGTCTTGTGCCGC
TCCTTGGCCAACGAGACCCATCAGCTGCGGAGGACGCTGACCGCCACCG
CCCACATGTGTCAGCATCTGGCCAAGTGTCTGGATGAACGACAGCATGC
ACAAAGGAATGTGGGGGAGAGAAGTCTTGACCAGTCGGAACACACAGAT
GGGCACACCTCTGTCCAGAGTGTATTGAGAAGTTGCAGGAAGAAAATC
GACTGTTAAAACAGAAGGTGACTCACGTTGAAGACCTCAATGCCAAGTG
GCAGCGCTACAACGCCAGCAGGGACGAATACGTGAGGGGGCTCCATGCG
CAGCTCAGGGGGCTGCAGATCCCCACGAGCCCGAGCTGATGAGGAAGG
AGATCTCCCGCTCAACAGACAGTTGGAAGAGAAAATAAATGACTGTGC
CGAAGTGAAGCAGGAGCTGGCGGCCTCCAGGACGGCCCGGGATGCTGCG
TTGGAGCGGGTGCAGATGCTGGAACAGCAGATTCTCGCTTACAAGGATG
ACTTCATGTCAGAAAGGGCCGATCGGGAACGGGCTCAAAGTAGGATTCA
AGAACCGGAGGAAAAGGTGCGCTCTTTGCTGCACCAGGTGCTCTGGAGA
CAGGATTCTCGAGAGCCAGACGCCGGCCGATTACGCTGGGAGCAAAA
CTGCCAAGTATTTGGCCGCCGACGCATTAGAGCTTATGGTGCTGGTGG
CTGGAGGCCTGGGACTGGGTCCCAGCAGCCAGAACCCCTGCAGAGGGC
GGGCATCCTGGCGCGGTCCAGAGAGGCCAGGGGGACCTTCAGTGCCCTC
ACTGCCTGCAGTGCTTCAGTGACGAGCAAGGGGAAGAGCTCCTCAGGCA
TGTGGCCGAGTGCTGCCAGtgagcggccgc