

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of ABIN2 D309N [1 - 429]

Enzyme description:- ABIN2 D309N [1 – 429]

Clone number:- DU 8638

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 75, 503.15 daltons

Average Mass 75, 550.80 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.00

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 D309N [1 – 429]

<u>Protein</u>	ABIN2 D309N [1 – 429]
<u>Clone number</u>	DU 8638
<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 VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVL FQG PLGSMSRDPGSGGWEEAP RAAAALCTLYHEAGQRLRRLQDQLAARDAL IARLRARLAAL EGDAAPSL VDALLEQVARFREQLRRQEGGAAEAQMRQEI ERLTERLEEKEREMOQLL SQPQHEREKEVVLLRRSMAEGERARAASDVLCRSLANETHQLRRTL TAT AHMCQH LAKCLDERQHAQRNVGERSPDQSEHTDGHTSVQSVIEKLQ EEN RLLKQKVTHVEDLN AKWQRYNASRDEYVRGLHAQLRGLQIPHEPELMRK EISRLNRQLEEKINDCAEVKQELAA SRTARDAALERVQMLEQQILAYKD NFMSEADRERAQSRIQELEEKVASLLHQVSWRQDSREPDAGRIHAGSK TAKYLAADALELMVPGGWRPGTGSQQPEPPAEGGHPGAVQRGQGDLOCP HCLQCF SDEQGEELLRHVAECCQ</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 D309N mutation. Residue D309 is equivalent to residue N540 of the fusion protein.</p>
<u>Protease cleavage</u>	PreScission (LEVL FQGP) 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
GCGCCGCTGCAGGACCAGCTCGCTGCCCGCGACGCCCTCATCGCTCGC
CTCCGCGCCCGCCTGGCCGCGCTGGAGGGGGACGCCGCGCCGTCCCTAG
TGGACGCGCTGCTGGAGCAGGTTGCGCGCTTCCGGGAGCAGCTGCGAAG
GCAGGAGGGCGGCGCCGCCGAGGCCAGATGCGCCAGGAAATTGAGAGG
CTGACTGAGCGACTAGAAGAAAAAGAGAGGGAGATGCAGCAGCTGCTGA
GCCAGCCCCAACACGAGCGAGAGAAGGAAGTCGTCCTGCTACGGAGGAG
CATGGCAGAAGGGGAGCGCGCCCGGGCCGCCAGTGACGTCCTGTGCCGC
TCCTTGGCCAACGAGACCCATCAGCTGCGGAGGACGCTGACCGCCACCG
CCCACATGTGTCAGCATCTGGCCAAGTGTCTGGATGAACGACAGCATGC
ACAAAGGAATGTGGGGGAGAGAAGTCCTGACCAGTCGGAACACACAGAT
GGGCACACCTCTGTCCAGAGTGTATTGAGAAGTTGCAGGAAGAAAATC
GACTGTTAAAACAGAAGGTGACTCACGTTGAAGACCTCAATGCCAAGTG
GCAGCGCTACAACGCCAGCAGGGACGAATACGTGAGGGGGCTCCATGCG
CAGCTCAGGGGGCTGCAGATCCCCACGAGCCCGAGCTGATGAGGAAGG
AGATCTCCCGGCTCAACAGACAGTTGGAAGAGAAAATAAATGACTGTGC
CGAAGTGAAGCAGGAGCTGGCGGCCTCCAGGACGGCCCGGGATGCTGCG
TTGGAGCGGGTGCAGATGCTGGAACAGCAGATTCTCGCTTACAAGGATA
ACTTCATGTCAGAAAGGGCCGATCGGGAACGGGCTCAAAGTAGGATTCA
AGAAGTGGAGGAAAAGGTCGCCTCTTTGCTGCACCAGGTGTCCTGGAGA
CAGGATTCTCGAGAGCCAGACGCCGGCCGGATTACGCTGGGAGCAAAA
CTGCCAAGTATTTGGCCGCCGACGCATTAGAGCTTATGGTGCTGGTGG
CTGGAGGCCTGGGACTGGGTCCCAGCAGCCAGAACCCCTGCAGAGGGC
GGGCATCCTGGCGCGGTCCAGAGAGGCCAGGGGGACCTTCAGTGCCCTC
ACTGCCTGCAGTGCTTCAGTGACGAGCAAGGGGAAGAGCTCCTCAGGCA
TGTGGCCGAGTGCTGCCAGtgagcggccgc