Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of ABIN2 D309N L327P [1 - 429]

Enzyme description:- ABIN1 D309N L327P [1 – 429]

Clone number:- DU 8640

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 75, 497.12 daltons
Average Mass 75, 534.75 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 L327P [1 – 429]

Protein ABIN2 D309N L327P [1 – 429]

Clone number DU 8640

Species Human

Accession number NM_024309.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSRDPGSGGWEEAP
RAAAALCTLYHEAGQRLRRLQDQLAARDALIARLRARLAALEGDAAPSL
VDALLEQVARFREQLRRQEGGAAEAQMRQEIERLTERLEEKEREMQQLL
SQPQHEREKEVVLLRRSMAEGERARAASDVLCRSLANETHQLRRTLTAT
AHMCQHLAKCLDERQHAQRNVGERSPDQSEHTDGHTSVQSVIEKLQEEN
RLLKQKVTHVEDLNAKWQRYNASRDEYVRGLHAQLRGLQIPHEPELMRK
EISRLNRQLEEKINDCAEVKQELAASRTARDAALERVQMLEQQILAYKD
NFMSERADRERAQSRIQEPEEKVASLLHQVSWRQDSREPDAGRIHAGSK
TAKYLAADALELMVPGGWRPGTGSQQPEPPAEGGHPGAVQRGQGDLQCP
HCLQCFSDEQGEELLRHVAECCQ**

Native sequence 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 and an L327P mutation. Residue D309 is equivalent to residue N540 of the fusion protein and residue L327 is equivalent to P558.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

ggatccATGTCCCGGGACCCGGGGTCGGGCGGCTGGGAGGAGGCCCCGC
GCGCAGCTGCCGCGCTCTGCACCCTGTACCACGAGGCCGGACAGCGGCT
GCGCCGCCTGCAGGACCAGCTCGCTGCCCGCGACGCCCTCATCGCTCGC
CTCCGCGCCCGCCTGGCCGCGCTGGAGGGGGACGCCGCGCCGTCCCTAG
TGGACGCGCTGCTGGAGCAGGTTGCGCGCTTCCGGGAGCAGCTGCGAAG
GCAGGAGGGCGGCGCCGCCGAGGCCCAGATGCGCCAGGAAATTGAGAGG
CTGACTGAGCGACTAGAAGAAAAAGAGAGGGAGATGCAGCAGCTGCTGA
GCCAGCCCCAACACGAGCGAGAGAAGGAAGTCGTCCTGCTACGGAGGAG
CATGGCAGAAGGGGAGCGCGCCCGGGCCGCCAGTGACGTCCTGTGCCGC
TCCTTGGCCAACGAGACCCATCAGCTGCGGAGGACGCTGACCGCCACCG
CCCACATGTGTCAGCATCTGGCCAAGTGTCTGGATGAACGACAGCATGC
ACAAAGGAATGTGGGGGAGAGAAGTCCTGACCAGTCGGAACACACAGAT
GGGCACACCTCTGTCCAGAGTGTTATTGAGAAGTTGCAGGAAGAAAATC
GACTGTTAAAACAGAAGGTGACTCACGTTGAAGACCTCAATGCCAAGTG
GCAGCGCTACAACGCCAGCAGGGACGAATACGTGAGGGGGCTCCATGCG
CAGCTCAGGGGGCTGCAGATCCCCCACGAGCCCGAGCTGATGAGGAAGG
AGATCTCCCGGCTCAACAGACAGTTGGAAGAGAAAATAAATGACTGTGC
CGAAGTGAAGCAGGAGCTGGCGGCCTCCAGGACGGCCCGGGATGCTGCG
TTGGAGCGGGTGCAGATGCTGGAACAGCAGATTCTCGCTTACAAGGATA
ACTTCATGTCAGAAAGGGCCGATCGGGAACGGGCTCAAAGTAGGATTCA
AGAACCGGAGGAAAAGGTCGCCTCTTTGCTGCACCAGGTGTCCTGGAGA
CAGGATTCTCGAGAGCCAGACGCCGGCCGGATTCACGCTGGGAGCAAAA
CTGCCAAGTATTTGGCCGCCGACGCATTAGAGCTTATGGTGCCTGGTGG
CTGGAGGCCTGGGACTGGGTCCCAGCAGCCAGAACCCCCTGCAGAGGGC
GGGCATCCTGGCGCGGTCCAGAGAGGCCAGGGGGACCTTCAGTGCCCTC
ACTGCCTGCAGTGCTTCAGTGACGAGCAAGGGGAAGAGCTCCTCAGGCA
TGTGGCCGAGTGCTGCCAGtgagcggccgc