Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of ABIN2 E313A [1 - 429]

Enzyme description:- ABIN2 E313A [1 – 429]

Clone number:- DU 8830

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic	75, 446.13 daltons
Average Mass	75, 493.74 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.99

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 E313A [1 – 429]

Protein	ABIN2 E313A [1 – 429]
Clone number	DU 8830
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** **DFMSARADRERAQSRIQELEEKVASLLHQVSWRQDSREPDAGRIHAGSK** **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 E313A mutation. Residue E313 is equivalent to residue A544 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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ggatccATGTCCCGGGACCCGGGGTCGGGCGGCTGGGAGGAGGCCCCGC
GCGCAGCTGCCGCGCTCTGCACCCTGTACCACGAGGCCGGACAGCGGCT
GCGCCGCCTGCAGGACCAGCTCGCTGCCCGCGACGCCCTCATCGCTCGC
CTCCGCGCCCGCCTGGCCGCGCTGGAGGGGGACGCCGCGCCGTCCCTAG
TGGACGCGCTGCTGGAGCAGGTTGCGCGCTTCCGGGAGCAGCTGCGAAG
GCAGGAGGGCGGCGCCGCCGAGGCCCAGATGCGCCAGGAAATTGAGAGG
CTGACTGAGCGACTAGAAGAAAAAGAGAGGGAGATGCAGCAGCTGCTGA
GCCAGCCCCAACACGAGCGAGAGAAGGAAGTCGTCCTGCTACGGAGGAG
CATGGCAGAAGGGGAGCGCGCCCGGGCCGCCAGTGACGTCCTGTGCCGC
TCCTTGGCCAACGAGACCCATCAGCTGCGGAGGACGCTGACCGCCACCG
CCCACATGTGTCAGCATCTGGCCAAGTGTCTGGATGAACGACAGCATGC
ACAAAGGAATGTGGGGGAGAGAAGTCCTGACCAGTCGGAACACACAGAT
GGGCACACCTCTGTCCAGAGTGTTATTGAGAAGTTGCAGGAAGAAAATC
GACTGTTAAAACAGAAGGTGACTCACGTTGAAGACCTCAATGCCAAGTG
GCAGCGCTACAACGCCAGCAGGGACGAATACGTGAGGGGGCTCCATGCG
CAGCTCAGGGGGCTGCAGATCCCCCACGAGCCCGAGCTGATGAGGAAGG
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CGAAGTGAAGCAGGAGCTGGCGGCCTCCAGGACGGCCCGGGATGCTGCG
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ACTTCATGTCAGCAAGGGCCGATCGGGAACGGGCTCAAAGTAGGATTCA
AGAACTGGAGGAAAAGGTCGCCTCTTTGCTGCACCAGGTGTCCTGGAGA
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CTGCCAAGTATTTGGCCGCCGACGCATTAGAGCTTATGGTGCCTGGTGG
CTGGAGGCCTGGGACTGGGTCCCAGCAGCCAGAACCCCCTGCAGAGGGC
GGGCATCCTGGCGCGGTCCAGAGAGGCCAGGGGGACCTTCAGTGCCCTC
ACTGCCTGCAGTGCTTCAGTGACGAGCAAGGGGAAGAGCTCCTCAGGCA
TGTGGCCGAGTGCTGCCAGtgagcggccgc
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