

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of ABIN3 E214A [1 - 325]

Enzyme description:- ABIN3 E214A [1 – 325]

Clone number:- DU 8777

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 66, 585.65 daltons

Average Mass 66, 628.21 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.78

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

ABIN3 E214A [1 – 325]

<u>Protein</u>	ABIN3 E214A [1 – 325]
<u>Clone number</u>	DU 8777
<u>Species</u>	Human
<u>Accession number</u>	NM_024873.5
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGSPGIPGSTRAAMAH FVQGTSRMIAAESSTEHKECAEPSTRKNLMNSLEQKIRCLEKQRKELLE VNQOWDQQFRSMKELYERKVAELKTKLDAAERFLSTREKDPHQQRKDD RQREDDRQRDLTRDLQREEKEKERLNEELHELKEENKLLKGKNTLANK EKEHYECEIKRLNKALQDALNIKCSFSEDCLRKSRVEFCHEEMRTEMEV LKQOVQIYEEDFKKARSDRERLNQEKEELQQINETSQSQNLNRLNSQIKA CQMEKEKLEKQLKQMYCPPCNCGLVFHLQDPWVPTGPGAVQKQREHPPD CQWYALDQLPPDVQHKANGLSSVKKVHP</p>
<u>Native sequence</u>	<p>Amino acids M1 – P325 (end) of human ABIN3. Residue M243 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 E214A mutation. Residue E214 is equivalent to residue A456 of the fusion protein.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVL FQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Not1</i> sites of pGEX6P-2

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Nucleotide
Sequence Of
Insert

gcggccgcgATGGCACATTTTGTACAGGGCACATCTAGAATGATTGCCG
CAGAAAGTTCTACGGAGCATAAAGAGTGTGCTGAACCATCAACAAGAAA
GAACTTGATGAATTCTCTTGAACAAAAGATAAGGTGTTTGGAAAAACAA
AGAAAAGAGCTCCTGGAAGTTAACCAGCAATGGGATCAGCAATTTAGAA
GTATGAAAGAGTTATATGAAAGAAAGGTAGCAGAGCTGAAGACGAAACT
GGACGCCGCGGAAAGATTCTCAGCACGCGGGAGAAGGATCCGCATCAG
AGGCAGAGAAAGGACGACAGGCAGAGAGAGGACGACAGGCAGCGCGACC
TGACCCGGGACCGGCTGCAGCGGGAGGAGAAGGAAAAGGAACGCCTAAA
TGAAGAATTACATGAATTGAAAGAAGAGAATAAACTTTTTAAAGGGAAAA
AATACTCTTGCGAACAAGGAAAAGGAACATTACGAATGTGAAATAAAAC
GCCTCAATAAGGCTCTTCAGGATGCCTTGAATATCAAGTGTTTCAATTTTC
CGAGGACTGTTTGAGGAAGTCTCGAGTGGAATCTGCCATGAGGAGATG
AGAACAGAAATGGAAGTTCTGAAGCAGCAGGTGCAATATACGAAGAAG
ACTTCAAAAAGGCACGATCGGATCGAGAGAGACTTAATCAAGAGAAAGA
GGAGCTACAGCAAATTAATGAAACTTCCAATCCCAGTTGAACAGGCTG
AATCCCAGATAAAAAGCTTGTCAGATGGAGAAAAGAAAAGCTAGAAAAGC
AATTA AACAGATGTATTGCCACCCTGTAAGTGGGCTTGGTTTTCCA
CCTGCAAGATCCATGGGTACCAACAGGCCCTGGAGCTGTGCAGAAGCAA
CGGGAGCACCCACCAGACTGTCAGTGGTATGCTCTTGACCAGCTTCCGC
CAGATGTACAACACAAGGCAAATGGTTTTATCCTCAGTAAAGAAAGTCCA
TCCGtaggcggccgc