

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of ABIN1 E476A [1 - 636]**

**Enzyme description:-** ABIN1 E476A [1 – 636]

**Clone number:-** DU 8750

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 98, 594.23 daltons

Average Mass 98, 656.40 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.07

**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**

**ABIN1 E476A [1 – 636]**

<b><u>Protein</u></b>	ABIN1 E476A [1 – 636]
<b><u>Clone number</u></b>	DU 8750
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_006058.4
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSMEGRGPYRIYDPG <b>GSVPSGEASAAFERLVKENSRLKEKMQGIKMLGELLEESQMEATRLRQK</b> <b>AEELVKDNELLPPSPSLGSFDPLAELTGKDSNVTASPTAPACPSDKPA</b> <b>PVQKPPSSGTSSEFEVVTPEEQNSPESSSHANAMALGPLPREDGNLMLH</b> <b>LQRLETTLSVCAEEP DHGQLFTHLGRMALEFNRLASKVHKNEQRTSILQ</b> <b>TLCEQLRKENEALKAKLDKGLEQRDQAAERLREENLELKKLLMSNGNKE</b> <b>GASGRPGSPKMEGTGKKAVAGQQQASVTAGKVPEVVALGAPEKKVKMLE</b> <b>QQRSELLEVNKQWDQHFRSMKQQYEQKITELRQKLADLQKQVTDLEAER</b> <b>EQKQRDFDRKLLAKSKIEMEETDKEQLTAEAKELRQKVLYLQDQLSPL</b> <b>TRQREYQEKEIQRLNKALEEALS IQTPPSSPPTAFGSPEGAGALLRKQE</b> <b>LVTQNELLKQOVKIFEEDFQARS DRERMNEEKEELKKQVEKLAQVTL</b> <b>SNAQLKAFKDEEKAREALRQQRKAKASGERYHVEPHPEHL CGAYPYAY</b> <b>PPMPAMVPHHGFEDWSQIRYPPPMAMEHPPPLPNSRLFHLPEYTWRLP</b> <b>CGGVRNPNQSSQVMDPPTARPT EPESPKNDRGPQ</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids M1 – Q636 (end) of human ABIN1. 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 E476A mutation. Residue E476 is equivalent to residue A713 of the fusion protein.</p>
<b><u>Protease cleavage</u></b>	PreScission (LEVL FQGP) residues 221 - 228
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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### Nucleotide Sequence Of Insert

ggatccATGGAAGGGAGAGGACCGTACCGGATCTACGACCCTGGGGGCA  
GCGTGCCCTCAGGAGAGGCATCCGCAGCTTTTGAGCGCCTAGTGAAGGA  
GAATTCCCGGCTGAAGGAAAAAATGCAAGGGATAAAGATGTTAGGGGAG  
CTTTTGGAAAGAGTCCCAGATGGAAGCGACCAGGCTCCGGCAGAAGGCAG  
AGGAGCTAGTGAAGGACAACGAGCTGCTCCCACCACCTTCTCCCTCCTT  
GGGCTCCTTCGACCCCTGGCTGAGCTCACAGGAAAGGACTCAAATGTC  
ACAGCATCTCCCACAGCCCCTGCATGCCCCAGTGACAAGCCAGCACCAG  
TCCAGAAGCCTCCATCCAGTGGCACCTCCTCTGAATTTGAAGTGGTCAC  
TCCTGAGGAGCAGAATTCACCAGAGAGCAGCAGCCATGCCAATGCGATG  
GCGCTGGGCCCCCTGCCCGTGAGGACGGCAACCTGATGCTGCACCTGC  
AGCGCCTGGAGACCACGCTGAGTGTGTGTGCCGAGGAGCCGGACCACGG  
CCAGCTCTTACCCACCTGGGCCGCATGGCCCTGGAGTTCAACCGACTG  
GCATCCAAGGTGCACAAGAATGAGCAGCGCACCTCCATTCTGCAGACCC  
TGTGTGAGCAGCTTCGGAAGGAGAACGAGGCTCTGAAGGCCAAGTTGGA  
TAAGGGCCTGGAACAGCGGGATCAGGCTGCCGAGAGGCTGCGGGAGGAA  
AATTTGGAGCTCAAGAAGTTGTTGATGAGCAATGGCAACAAAGAGGGTG  
CGTCTGGGCGCCAGGCTCACCGAAGATGGAAGGGACAGGCAAGAAGGC  
AGTGGCTGGACAGCAGCAGGCTAGTGTGACGGCAGGTAAGGTCCCAGAG  
GTGGTGGCCTTGGGCGCACCCGAGAAGAAGGTGAAGATGCTGGAGCAGC  
AGCGCAGTGAGCTGCTGGAAGTGAACAAGCAGTGGGACCAGCATTTCCG  
GTCCATGAAGCAGCAGTATGAGCAGAAGATCACTGAGCTGCGTCAGAAG  
CTGGCTGATTTGCAGAAGCAGGTGACTGACCTGGAGGCCGAGCGGGAGC  
AGAAGCAGCGTGACTTTGACCGCAAGCTCCTCCTGGCCAAGTCCAAGAT  
TGAAATGGAGGAGACCGACAAGGAGCAGCTGACAGCAGAGGCCAAGGAG  
CTGCGCCAAAAGGTCAAGTACCTGCAGGATCAGCTGAGCCCCTCACCC  
GACAGCGTGAGTACCAGGAAAAGGAGATCCAGCGGCTCAACAAGGCCCT  
GGAGGAAGCACTGAGCATCCAAACCCCGCCATCATCTCCACCAACAGCA  
TTTGGGAGCCCAGAAGGAGCAGGGGCCCTCCTAAGGAAACAGGAGCTGG  
TCACGCAGAATGAGTTGCTGAAACAGCAGGTGAAGATCTTCGAGGAGGA  
CTTCCAGAGGGCGCGCAGTGATCGTGAGCGCATGAATGAGGAGAAGGAA  
GAGCTGAAGAAGCAAGTGGAGAAGCTGCAGGCCCAGGTACCCCTGTCAA  
ATGCCCAGCTAAAAGCATTCAAAGATGAGGAGAAGGCAAGAGAAGCCCT  
CAGACAGCAGAAGAGGAAAAGCAAAGGCCCTCAGGAGAGCGTTACCATGTG  
GAGCCCCACCCAGAACATCTCTGCGGGCCTACCCCTACGCCTACCCGC  
CCATGCCAGCCATGGTGCCACACCATGGCTTCGAGGACTGGTCCCAGAT  
CCGCTACCCCTCCCCCATGGCCATGGAGCACCCGCCCCCACTCCCC  
AACTCGCGCCTCTTCCATCTGCCGGAATACACCTGGCGTCTACCTTGTG  
GAGGGGTTCGAAATCCAAATCAGAGCTCCCAAGTGATGGACCCCTCCCAC  
AGCCAGGCCTACAGAACCAGAGTCTCCAAAAAATGACCGTGAGGGGCCT  
CAGtgagcggccgc

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