Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Autophagy related 13 [1 – 480]

Enzyme description:- ATG13 [1 – 480]

Clone number:- DU 30086

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 79, 595.78 daltons
Average Mass 79, 646.77 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.12

Purity:- 85 %

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- –70 °C

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Clone Data Sheet

Autophagy related 13 [1 – 480]

Protein	ATG13 [1 - 480]
Clone number	DU 30086
Species	Human
Accession number	NM_014741.4
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**METDLNSQDRKDLDKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPEVTHEAKKALAGQLPAVGRSMCVEISLKTSEGDSMELEIWCLEMNEKCDKEIKVSYTVYNRLSLLLKSLLAITRVTPAYRLSRKQGHEYVILYRIYFGEVQLSGLGEGFQTVRVGTVGTPVGTITLSCAYRINLAFMSTRQFERTPPIMGIIIDHFVDRPYPSSSPMHPCNYRTAGEDTGVIYPSVEDSQEVCTTSFSTSPPSQLMVPGKEGGVPLAPNQPVHGTQADQERLATCTPSDRTHCAATPSSSEDTETVSNSSEGRASPHDVLETIFVRKVGAFVNKPINQVTLTSLDIPFAMFAPKNLELEDTDPMVNPPDSPETESPLQGSLHSDGSSGGSSGNTHDDFVMIDFKPAFSKDDILPMDLGTFYREFQNPPQLSSLSIDIGAQSMAEDLDSLPEKLAVHEKNVREFDAFVETLQ**
Native sequence	Amino acids M1 – Q480 of human ATG13. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX-6P-1

Nucleotide sequence of insert

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ggatccATGGAAACTGATCTCAATTCCCAGGACAGAAAGGACCTG
GACAAGTTTATTAAATTTTTTGCCCTCAAGACTGTCCAAGTGATT
GTCCAGGCTCGGCTTGGTGAAAAGATTTGCACTCGTTCATCATCT
TCTCCAACGGGTTCAGATTGGTTCAACTTAGCAATCAAAGACATC
CCAGAGGTTACACATGAAGCAAAGAAGGCACTGGCAGGACAGCTG
CCTGCAGTCGGGAGGTCCATGTGTGTGGAGATTTCACTTAAGACT
TCTGAGGGAGATTCCATGGAGCTGGAAATATGGTGTCTTGAAATG
AATGAAAAGTGTGATAAAGAAATCAAAGTTTCCTACACGGTGTAC
AACAGACTGTCATTGCTGCTGAAGTCCCTTCTTGCTATAACTAGG
GTGACACCAGCCTATAGGCTCTCCAGGAAACAAGGGCATGAATAT
GTCATATTATACAGGATATATTTTGGAGAAGTTCAGCTGAGTGGC
TTAGGAGAAGGCTTCCAGACAGTTCGTGTTGGGACAGTGGGCACC
CCTGTGGGCACCATCACTCTTTCTTGTGCTTACAGAATTAACTTG
GCATTCATGTCTACCAGGCAATTTGAGAGGACCCCACCTATCATG
GGGATTATTATTGATCACTTTGTGGACCGTCCCTATCCCAGCTCC
TCTCCCATGCACCCCTGCAATTACAGAACTGCTGGTGAGGACACT
GGAGTAATATACCCGTCTGTAGAAGACTCTCAAGAAGTGTGTACC
ACCTCTTTTTCCACCTCCCCACCATCCCAGCTGATGGTTCCTGGG
AAGGAAGGTGGGGTACCCCTTGCTCCCAACCAGCCTGTCCATGGT
ACCCAGGCTGACCAGGAGAGACTGGCAACCTGCACCCCTTCTGAC
AGAACCCACTGTGCTGCCACACCCTCCAGTAGTGAGGATACTGAA
ACCGTATCAAACAGCAGTGAGGGACGGGCCTCCCCTCACGATGTC
TTGGAGACCATCTTTGTCCGAAAAGTGGGGGCTTTTGTCAACAAA
CCCATTAACCAGGTGACCCTGACGAGTTTGGATATACCCTTTGCC
ATGTTTGCTCCCAAGAATTTGGAGCTGGAGGATACCGATCCAATG
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AGCCTGCACTCAGATGGCTCCAGCGGGGGCAGCAGTGGCAATACC
CATGATGACTTTGTTATGATAGACTTTAAACCAGCTTTTTCTAAA
GATGACATTCTTCCGATGGACCTGGGGACCTTCTATCGGGAGTTT
CAGAACCCACCTCAGCTGAGCAGCCTCTCCATAGATATTGGAGCA
CAGTCCATGGCTGAAGACTTGGACTCATTACCAGAGAAGCTGGCT
GTGCATGAGAAGAATGTCCGCGAGTTTGATGCCTTTGTGGAAACC
CTGCAGtaagcggccgc
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