Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of ATG5 [1 – 275]

Enzyme description:- ATG5 [1 - 275]

Clone number:- DU 40013

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 59, 749.08 daltons
Average Mass 59, 787.83 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.62

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

ATG5 [1 - 275]

Protein	ATG5 [1 - 275]
Clone number	DU 40013
Species	Human
Accession number	NM_004849.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDHPPKSDENLYFQGGS**MTDDKDVLRD** **VWFGRIPTCFTLYQDEITEREAEPYYLLLPRVSYLTLVTDKVKKHFQKV** **MRQEDISEIWFEYEGTPLKWHYPIGLLFDLLASSSALPWNITVHFKSFP** **EKDLLHCPSKDAIEAHFMSCMKEADALKHKSQVINEMQKKDHKQLWMGL** **QNDRFDQFWAINRKLMEYPAEENGFRYIPFRIYQTTTERPFIQKLFRPV** **AADGQLHTLGDLLKEVCPSAIDPEDGEKKNQVMIHGIEPMLETPLQWLS** **EHLSYPDNFLHISIIPQPTD**
Native sequence	Amino acids M1 – D275 (end) of human ATG5. Residue M236 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	TEV protease (ENLYFQG) residues 227 – 233
Cloning sites	*Bam*H1 and *Sal*1 sites of pGEX6P-1

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Nucleotide Sequence of insert

ggatccATGACAGATGACAAAGATGTGCTTCGAGATGTGTGGTTTGGACGAATTCCAACTTGTTTC
ACGCTATATCAGGATGAGATAACTGAAAGGGAAGCAGAACCATACTATTTGCTTTTGCCAAGAGTA
AGTTATTTGACGTTGGTAACTGACAAAGTGAAAAAGCACTTTCAGAAGGTTATGAGACAAGAAGAC
ATTAGTGAGATATGGTTTGAATATGAAGGCACACCACTGAAATGGCATTATCCAATTGGTTTGCTA
TTTGATCTTCTTGCATCAAGTTCAGCTCTTCCTTGGAACATCACAGTACATTTTAAGAGTTTTCCA
GAAAAAGACCTTCTGCACTGTCCATCTAAGGATGCAATTGAAGCTCATTTTATGTCATGTATGAAA
GAAGCTGATGCTTTAAAACATAAAAGTCAAGTAATCAATGAAATGCAGAAAAAAGATCACAAGCAA
CTCTGGATGGGATTGCAAAATGACAGATTTGACCAGTTTTGGGCCATCAATCGGAAACTCATGGAA
TATCCTGCAGAAGAAAATGGATTTCGTTATATCCCCTTTAGAATATATCAGACAACGACTGAAAGA
CCTTTCATTCAGAAGCTGTTTCGTCCTGTGGCTGCAGATGGACAGTTGCACACACTAGGAGATCTC
CTCAAAGAAGTTTGTCCTTCTGCTATTGATCCTGAAGATGGGGAAAAAAAGAATCAAGTGATGATT
CATGGAATTGAGCCAATGTTGGAAACACCTCTGCAGTGGCTGAGTGAACATCTGAGCTACCCGGAT
AATTTTCTTCATATTAGTATCATCCCACAGCCAACAGATtgagtcgac