

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of ATG101 [1 – 218]

Enzyme description:- ATG101 [1 - 218]

Clone number:- DU 45655

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 51, 793.34 daltons

Average Mass 51, 826.80 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.77

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

ATG101 [1 - 218]

<u>Protein</u>	ATG101 [1 - 218]
<u>Clone number</u>	DU 45655
<u>Species</u>	Human
<u>Accession number</u>	NM_021934.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMNCRSEVLEVSVEG RQVEEAMLAVLHTVLLHRSTGKFFHYKKEGTYSIGTVGTQDVDCDFIDFT YVRVSSEELDRALRKVVGEFKDALRNSGGDGLGQMSLEFYQKKKSRWPF SDECIPWEVWTVKVHVVALATEQERQICREKVGEKLCEKIINIVEVMNR HEYLPKMPTQSEVDNVFDTGLRDVQPYLYKISFQITDALGTSVTTTMR LIKDTLAL
<u>Native sequence</u>	Amino acids M1 – L218 (end) of human ATG101. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (LEVL FQGP) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pGEX6P-3

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Nucleotide Sequence of insert

ggatccATGAACTGTCGCTCGGAGGTGCTGGAGGTGTCGGTGGAGGGCGGCAGGTGGAGGAGGCC
ATGCTGGCTGTGCTGCACACGGTGCTTCTGCACCGCAGCACAGGCAAGTTCCACTACAAGAAGGAG
GGCACCTACTCCATTGGCACCGTGGGCACCCAGGATGTTGACTGTGACTTCATCGACTTCACTTAT
GTGCGTGTCTCTTCTGAGGAACTGGATCGTGCCCTGCGCAAGGTTGTTGGGGAGTTCAAGGATGCA
CTGCGCAACTCTGGTGGCGATGGGCTGGGGCAGATGTCCTTGGAGTTCTACCAGAAGAAGAAGTCT
CGCTGGCCATTCTCAGACGAGTGCATCCCATGGGAAGTGTGGACGGTCAAGGTGCATGTGGTAGCC
CTGGCCACGGAGCAGGAGCGGCAGATCTGCCGGGAGAAGGTGGGTGAGAAACTCTGCGAGAAGATC
ATCAACATCGTGGAGGTGATGAATCGGCATGAGTACTTGCCCAAGATGCCCACACAGTCGGAGGTG
GATAACGTGTTTGACACAGGCTTGCGGGACGTGCAGCCCTACCTGTACAAGATCTCCTTCCAGATC
ACTGATGCCCTGGGCACCTCAGTCACCACCACCATGCGCAGGCTCATCAAAGACACCCTTGCCCTC
tgagaattc