

*Division of Signal Transduction Therapy*

**Standard Operation Procedure**

**Preparation of GST-ATG4b**

<b><u>Enzyme description:-</u></b>	GST-ATG4b
<b><u>Clone number:-</u></b>	DU40010
<b><u>Source:-</u></b>	BL21 Recombinant
<b><u>Tag:-</u></b>	N-terminal
<b><u>Purification method:-</u></b>	GSH sepharose
<b><u>Expression level:-</u></b>	4 mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	71063 Da
Average Mass	71647 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	5.26
<b><u>Purity:-</u></b>	80%
<b><u>Enzyme storage buffer:-</u></b>	
50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<b><u>Storage temperature:-</u></b>	-80°C

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**Clone Data Sheet**

**GST-ATG4b**

<b><u>Protein</u></b>	GST-ATG4b
<b><u>Synonyms</u></b>	APG4B, AUTL1
<b><u>Clone Number</u></b>	DU40010
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: Q9Y4P1 DNA: NM013325.4
<b><u>Tags</u></b>	N-terminal GST tag
<b><u>Amino acid sequence of expressed protein</u></b>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFP NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGV SRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDAL DVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLOGWQATFGG GDHPPKSDHPPKSDENLYFQGGSM <b>DAATLT</b> YD <b>TLRFAEFEDFPETSEPVWILG</b> <b>RKYSIFTEKDEILSDVASRLWFTYRKNFPAIGGTGPTSDTGWGCMLRCGQMI</b> <b>AQALVCRHLGRDWRWTQRKRQ</b> PDSYF <b>SVLNAFIDRKDSYYSIHQIAQMGV</b> GEG <b>KSIGQWYGPNTVAQVLK</b> KLAVFD <b>TWSSLAVHIAMDNTVVMEEIRRLCRTSVPC</b> <b>AGATAFPADSDRHCNGFPAGAEVTNRPSWRPLVLLIPLRLGLTDINEAYVET</b> <b>LKHC</b> FMPQSLGVIGG <b>KPNSAHYFIGYVGEELIYLDPHTTQPAVEPTDGC</b> FIP <b>DESFHCQHPPCRMSIAELDPSIAVGFFCKTEDDFNDWCQQVKKLSLLGGALPM</b> <b>FELVEQQPSHLACPDVNLNLSLDSSDVERLERFFDSEDEDFEILSL</b>
<b><u>Native sequence</u></b>	in bold
<b><u>Protease cleavage</u></b>	TEV site underlined
<b><u>Cloning sites</u></b>	BamH1 / Not1

**DNA sequence of  
insert**

GGATCCATGGACGCAGCTACTCTGACCTACGACACTCTCCGGTTTGCTGAGTT  
TGAAGATTTTCCTGAGACCTCAGAGCCCGTTTGGATACTGGGTAGAAAATACA  
GCATTTTTCACAGAAAAGGACGAGATCTTGTCTGATGTGGCATCTAGACTTTGG  
TTTACATACAGGAAAACTTCCAGCCATTGGGGGGACAGGCCCCACCTCGGA  
CACAGGCTGGGGCTGCATGCTGCGGTGTGGACAGATGATCTTTGCCCAAGCCC  
TGGTGTGCCGGCACCTAGGCCGAGATTGGAGGTGGACACAAAGGAAGAGGCAG  
CCAGACAGCTACTTCAGCGTCCCAACGCATTCATCGACAGGAAGGACAGTTA  
CTACTCCATTACACAGATAGCGCAAATGGGAGTTGGCGAAGGCAAGTCCATAG  
GCCAGTGGTACGGGCCCAACACTGTCGCCCAGGTCCTGAAGAAGCTTGCTGTC  
TTCGATACGTGGAGCTCCTTGGCGGTCCACATTGCAATGGACAACACTGTTGT  
GATGGAGGAAATCAGAAGGTTGTGCAGGACCAGCGTTCCCTGTGCAGGCGCCA  
CTGCGTTTCCTGCAGATTCCGACCGGCACTGCAACGGATTCCCTGCCGGAGCT  
GAGGTCACCAACAGGCCGTCGCCATGGAGACCCCTGGTACTTCTCATTCCCCT  
GCGCCTGGGGCTCACGGACATCAACGAGGCCACGTGGAGACGCTGAAGCACT  
GCTTCATGATGCCCCAGTCCCTGGGCGTCATCGGAGGGAAGCCCAACAGCGCC  
CACTACTTCATCGGCTACGTTGGTGAGGAGCTCATCTACCTGGACCCCCACAC  
CACGCAGCCAGCCGTGGAGCCCACTGATGGCTGCTTCATCCCGGACGAGAGCT  
TCCACTGCCAGCACCCGCCGTGCCGCATGAGCATCGCGGAGCTTGACCCGTCC  
ATCGCTGTGGGGTTTTTCTGTAAGACTGAAGATGACTTCAATGATTGGTGCCA  
GCAAGTCAAAAAGCTGTCTCTGCTCGGAGGTGCCCTGCCCATGTTTGGAGCTGG  
TGGAGCAGCAGCCTTACATCTGGCCTGCCCGACGTCCTGAACCTGTCCCTA  
GATTCTTCTGATGTAGAGCGACTGGAAAGATTCTTCGACTCAGAAGATGAAGA  
CTTTGAAATCCTGTCCCTTTGAGCGGCCGC