

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-LC3b

Enzyme description:- GST-LC3b 1-120 = mature full length

Clone number:- DU40082

Source:- human recombinant

Tag:- GST-

Purification method:- GSH-Sepharose

Expression system:- E.coli

Calculated molecular mass:-

Monoisotopic 41443 Da

Average Mass 41469 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.57

Purity:- 90%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80°C

Assay:-

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Clone Data Sheet

GST-LC3b

<u>Protein</u>	GST-LC3b 1-120 = mature full length
<u>Synonyms</u>	ATG8F; LC3B; MAP1A/1BLC3
<u>Clone Number</u>	DU40082
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_073729 DNA: NM_022818.4
<u>Tags</u>	GST-
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFE LGLFEPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISM LEGAVLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKT YLN GDHVTHPDMFLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPO IDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDHPPKSDENLYFQGG MPSEKTFKQRRTFEQRVEDVRLIREQHPTKIPVIERYKGEKQLPVL DKTKFLVPDHVNMSELIKIIRRRLQLNANQAFLLVNGHSMVSVSTP ISEVYESEKDEDGFLYMYASQETFG
Native sequence	LC3B is normally expressed as a 125aa precursor and cleaved to a mature 120 residue form (in bold).
Protease cleavage	TEV site underlined
Cloning sites	BamHI / NotI
<u>DNA sequence of expression cassette</u>	ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACC CACTCGACTTCTTTTGGAAATATCTTGAAGAAAATATGAAGAGCATT TGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAGTTTGAA TTGGGTTTGGAGTTTCCCAATCTTCTTATTATATTGATGGTGATGT TAAATTAACACAGTCTATGGCCATCATA CGTTATATAGCTGACAAGC ACAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATG CTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTCGAGAATTGC ATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGC TACCTGAAATGCTGAAAATGTTCTGAAGATCGTTTATGTCATAAAACA TATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGA CGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCTGGATGCGT TCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCACAA ATTGATAAGTACTTGAATCCAGCAAGTATATAGCATGGCCTTTGCA GGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGG ATCATCCTCCAAAATCGGATGAAAACCTGTATTTTTCAGGGCggatcc atgccgtcggagaagaccttcaagcagcgccgcaccttcgaacaaag agtagaagatgtccgacttatcggagagcagcatccaaccaaatacc cggtgataatagaacgatacaagggtgagaagcagcttcctgttctg gataaaacaaagttccttgtagctgacctgacctgcaacatgagtgagct catcaagataattagaaggcgcttacagctcaatgctaatacaggcct tcttctgtttggtgaacggacacagcatgggtcagcgtctccacacca

atctcagaggtgtatgagagtgagaaagatgaagatggattcctgta
catggtctatgcctcccaggagacgttcgggtaa