Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of LC3b

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

Clone number:- DU40082

Source:- human recombinant

Tag:- cleaved from GST-

Purification method:- GSH-Sepharose, protease treatment

Expression system:- E. coli

Calculated molecular mass:-

Monoisotopic	14321 Da
Average Mass	14330 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.02

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80ºC

Assay:-

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Clone Data Sheet

LC3b

Protein	LC3b 1 -120 = mature full length
Synonyms	ATG8F; LC3B; MAP1A/1BLC3
Clone Number	DU40082
Species	Human
Accession Number	Protein: Q9GZQ8 DNA: NM_022818.4
Tags	cleaved from GST-
Aminoacid sequence of the expressed protein	GGS**MPSEKTFKQRRTFEQRVEDVRLIREQHPTKIPVIIERYKGEKQL PVLDKTKFLVPDHVNMSELIKIIRRRLQLNANQAFFLLVNGHSMVSV STPISEVYESEKDEDGFLYMVYASQETFG**
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

DNA sequence of expression cassette

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ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACC
CACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATT
TGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAA
TTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGT
TAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGC
ACAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATG
CTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGC
ATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGC
TACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACA
TATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGA
CGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCTGGATGCGT
TCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAA
ATTGATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCA
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ATCATCCTCCAAAATCGGATGAAAACCTGTATTTTCAGGGCggatcc
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