

## *Division of Signal Transduction Therapy*

### Standard Operation Procedure

#### Preparation of His-LC3b

**Enzyme description:-** His-LC3b 1-20 = mature full length

**Clone number:-** DU40076

**Source:-** human recombinant

**Tag:-** His-

**Purification method:-** Ni<sup>2+</sup>-NTA-Sepharose

**Expression system:-** E.coli

**Calculated molecular mass:-**

Monoisotopic 16912 Da

Average Mass 16923 Da

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 7.7

**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**

**His-LC3b**

<b><u>Protein</u></b>	His-LC3b 1-120 = full length
<b><u>Synonyms</u></b>	ATG8F; LC3B; MAP1A/1BLC3
<b><u>Clone Number</u></b>	DU40076
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: NP_073729      DNA: NM_022818.4
<b><u>Tags</u></b>	N-terminal His-
Aminoacid sequence of the expressed protein	MGSSHHHHHHSSGENLYFQGHMLGSM <b>PSEKTFKQRRTFEQRVEDVRL IREQHPTKIPVI IERYKGEKQLPVL</b> DKTKFLVPDHVN <b>MSELIKI IRR RLQLNANQAFFLLVNGHSMVSVSTP ISEVYESEKDEDGFLY</b> MVYASQ <b>ETFG</b>
Native sequence	LC3B is normally expressed as a 125aa precursor and cleaved to a mature 120 residue form ( <b>in bold</b> ).
Protease cleavage	TEV site underlined
Cloning sites	BamHI / NotI
<b><u>DNA sequence of expression cassette</u></b>	ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCGAAAACCT GTATTTTCAGGGCCATATGCTGGGATCC <b>atgccgtcggagaagacct</b> <b>tcaagcagcgcgcaccttcgaacaaagagtagaagatgtccgactt</b> <b>atcgagagcagcatccaacaaaatcccggtgataatagaacgata</b> <b>caagggtgagaagcagcttcctgttctggataaaacaaagttccttg</b> <b>tacctgaccatgtcaacatgagtgagctcatcaagataattagaagg</b> <b>cgcttacagctcaatgctaatacaggccttctcctgttggtgaacgg</b> <b>acacagcatggtcagcgtctccacaccaatctcagaggtgatgaga</b> <b>gtgagaaagatgaagatggattcctgtacatggtctatgcctccag</b> <b>gagacgttcgggtaa</b>