LC3b [GST-tagged]

Modifying Protein

Alternate Names: Microtubule-Associated Protein 1, Light Chain 3, Beta; MAP1LC3B

Cat. No.	**60-0111-500**	Quantity:	500 µg
Lot. No.	**30098**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

Background

The enzymes of the autophagy pathway play a pivotal role in the degradation of cytoplasmic constituents and organelles. Structures known as autophagosomes sequester portions of the cytoplasm which are degraded by the lysosome and recycled back into the cell. (Kuma *et al.* 2004). Three classes of enzymes are involved in autophagy; E1-like activating enzymes (E1s), E2-like conjugating enzymes (E2s) and ubiquitin-like proteins (ubls). Microtubule Associated Protein 1, Light Chain 3 beta, MAP1LC3b or LC3b is a member of the Ubl family and cloning of the human gene was first described by He *et al.* (2003). The C-terminal of LC3b is cleaved by ATG4B C-terminal to Gly120 *in vitro* and this has been shown to be required for the formation of intermediates with the ubiquitin-like activating enzyme ATG7 (Tanida *et al.* 2004). A proteomic analysis of the autophagosome interaction network was performed on a human cell line undergoing a basal level of autophagy. This study revealed that six of the ATG8 homologues (including LC3b) interacted with a cohort of 67 proteins in which there was frequent involvement with a conserved ATG8 surface region shown previously to interact with LC3 interacting regions in partner proteins (Behrends *et al.* 2010).

References:

Behrends C, Sowa ME, Gygi SP, Harper JW (2010) Network organization of the human autophagy system. *Nature* **466**, 68-76.

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 500 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: 41.48 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

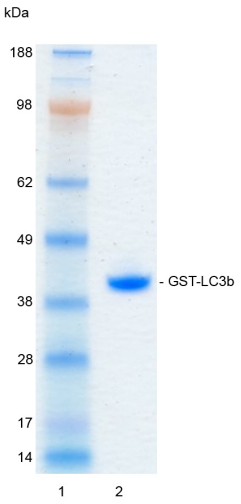
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATF
GGGDHPPKSDHPPKSDENLYFQGGS***M***PSEK
TFKQRRTFEQRVEDVRLIREQHPTKIPVI
IERYKGEKQLPVLDKTKFLVPDHVNMSELIKI
IRRRLQLNANQAFFLLVNGHSMVSVSTPI
SEVYESEKDEDGFLYMVYASQETFG

Tag (**bold text**): N-terminal GST
Protease cleavage site: TEV (ENLYF▼QG)
LC3b (regular text): Start ***bold italics*** (amino acid residues 1-120)
Accession number: NP_073729.1

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-LC3b



Protein Identification:
Confirmed by mass spectrometry.

E1 Thioester LC3b Loading Assay:
The activity of LC3b was validated by loading LC3b onto the active cysteine of His-ATG7. Incubation of the His-ATG7 enzyme in the presence of LC3b and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the LC3b/His-ATG7 thioester bond to the reducing agent DTT was confirmed.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

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He H, Dang Y, Dai F, Guo Z, Wu J, et al. (2003) Post-translational modifications of three members of the human MAP1LC3 family and detection of a novel type of modification for MAP1LC3B. *J Biol Chem* **278**, 29278-29287.

Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, *et al.* (2004) The role of autophagy during the early neonatal starvation period. *Nature* **432**, 1032-1036.

Tanida I, Ueno T, Kominami E (2004) Human light chain 3/MAP1L-C3B is cleaved at its carboxyl-terminal Met121 to expose Gly120 for lipidation and targeting to autophagosomal membranes. *J Biol Chem* **279**, 47704-47710.

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Lot-specific COA version tracker: v1.0.0