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Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier

Forked from Expression and purification protocol of the Human (Homo sapiens) mCherry-LC3B Ubiquitin-like modifier

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We use this protocol and it's working

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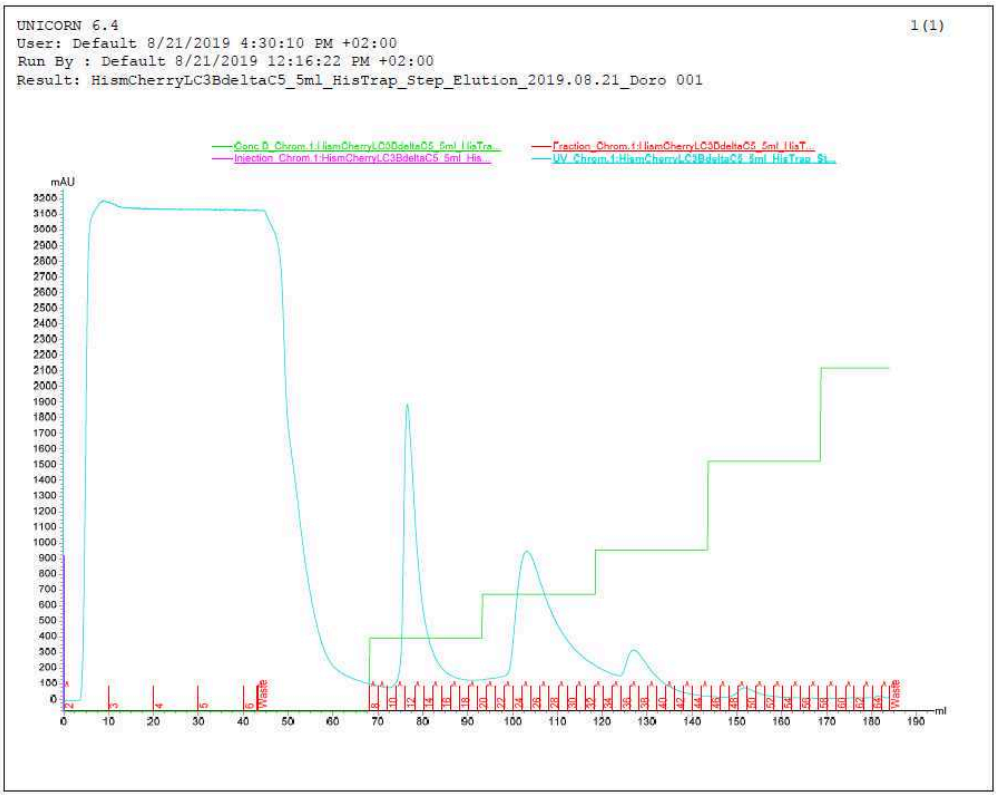
Abstract

This protocol describes expression and purification procedures for obtaining mCherry-tagged human recombinant Ubiquitin-like modifier LC3B (MAP1LC3B, Microtubule-associated proteins 1A/1B light chain 3B) lacking five C-terminal
amino acids to allow in vitro protein conjugation to target PE, Phosphatidyl Ethanolamine.

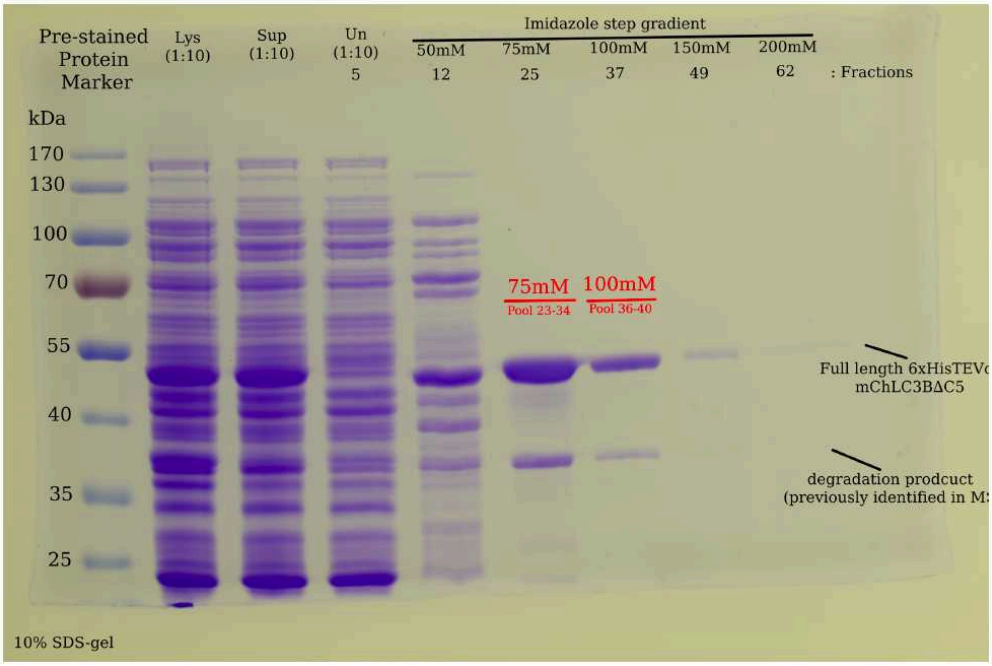
Image Attribution

Guidelines

Affinity purification

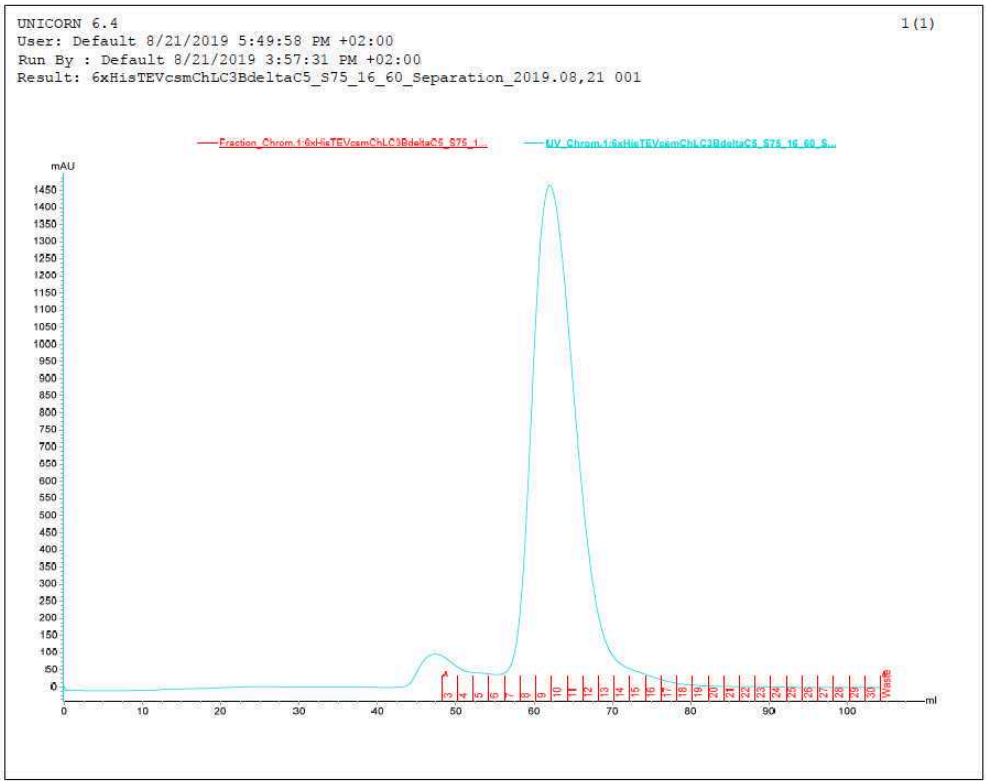


Chromatograph of His-tag affinity purification for mCherry-hLC3B.

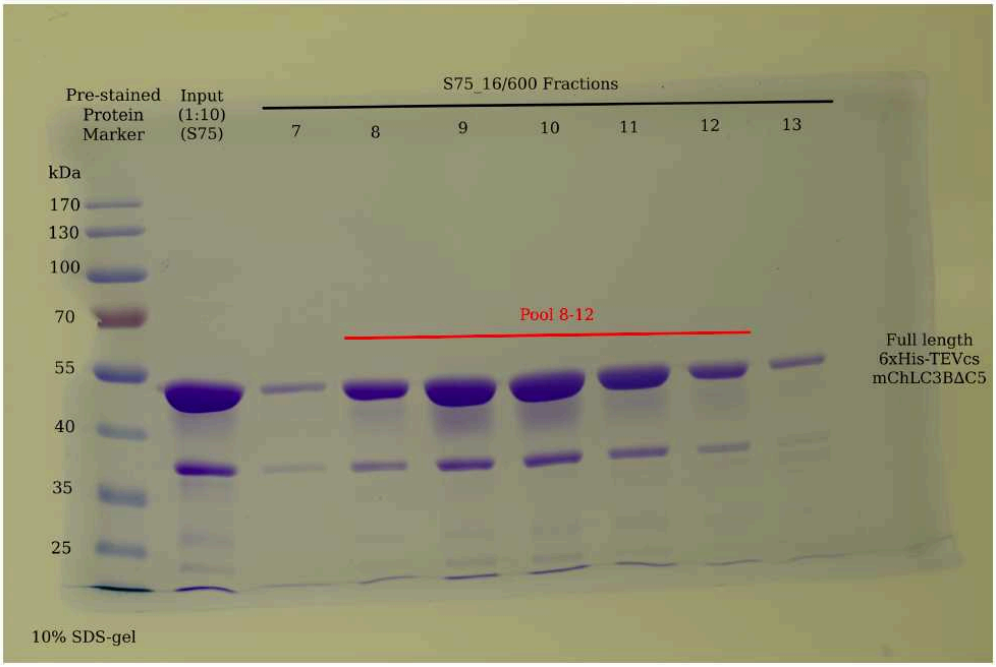


Coomassie BB stained gel of His-tag affinity purification for mCherry-hLC3B.

Size Exclusion Chromatography



Chromatograph of Size Exclusion purification for mCherry-hLC3B.



Coomassie BB stained gel of Size Exclusion Chromatography purification for mCherry-hLC3B.

Materials

Materials and Reagents

- Escherichia coli Rosetta pLyss cells
- Luria Bertani (LB) medium with antibiotics (final conc. 50µg/ml Ampicillin, 34µg/ml Chloramphenicol)
- IPTG (isopropyl-b-d-thiogalactopyranoside)
- 37°C shaker incubator
- sterile flasks/sterile pipettes
- tip sonicator

- **Lysis Buffer:** 50mM Hepes pH=7.5, 300mM NaCl, 10mM Imidazole, 2mM $MgCl_2$, 2mM β-mercaptoethanol, 1mM Pefablock, Complete Protease Inhibitors (EDTA-free CIP tablet, Roche), DNAse (Sigma).
- **Buffer A:** 50mM Hepes pH=7.5, 300mM NaCl, 10mM Imidazole (filtered and degassed) + 1mM β-mercaptoethanol;
- **Buffer B:** 50mM Hepes pH=7.5, 300mM NaCl, 300mM Imidazole (filtered and degassed) + 1mM β-mercaptoethanol;
- **Size Exclusion Chromatography (SEC) Buffer:** 25mM Hepes pH=7.5, 150mM NaCl, 1mM DTT (Dithiothreitol).

Note: all purification buffers are filtered and degassed. Reducing agents (β-mercaptoethanol and Dithiothreitol) are added after degassing step.

Columns:- HT 5ml column (GE Healthcare)
- S75_16/60 (GE Healthcare)

Gels:10% SDS-PAGE

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Before start

General information

Insert: Homo sapiens LC3B, **NP_073729.1**; Expression system: E.Coli Rosetta pLyss; plasmid origin: Sascha Martens Lab, Addgene 169168, lab internal construct database number SMC948; backbone: pET-Duet1; plasmid resistance: Ampicillin; tags & cleavage sites: N-term 6xHis, followed by Tobacco Etch Virus (TEV) cleavage site, mCherry tag, LC3B ORF. Ext coeff: 41830 M^{-1} cm^{-1} , MW 47,89 kDa.

Protein Expression

16h 15m

1 Transform plasmid DNA (Addgene_190237) into E.Coli Rosetta pLyss cells and plate on Ampicillin LB agar plate for Overnight at 37 °C .

2 The following day, inoculate a 5 mL LB + Amp/Cam pre-culture with 1-2 colonies and grow Overnight at 37 °C shaking.

3 The following day, use 5 mL pre-culture to inoculate 1 L LB medium + Amp/Cam at 37 °C until an OD_{600} (Optical Density) of 0.6 is reached.

4 Induce protein expression with [M] 400 micromolar (µM) IPTG and grow for a further 16:00:00 at 18 °C shaking.

16h

5 Pellet cells at 4000 rpm, 4°C, 00:15:00 in a Sorvall RC6+ centrifuge (Thermo Scientific), discard supernatant and resuspend pellets in ice cold lysis buffer (25 ml/1 lt culture).

15m

6 Flash freeze resuspended pellets in liquid nitrogen and store at -80 °C until purification.

Protein Purification

7 Perform His-Trap affinity purification followed by Size Exclusion Chromatography.

Purification of LC3B Ubiquitin-like modifier

30m 30s

8 Cells are lised via freeze/thaw cycles and sonication: thaw pellet corresponding to 1 L culture by freeze/thawing in Room temperature water bath. All following steps are to be executed at 4 °C or On ice .

9 Lyse cells by sonicating them with an immersion Tip Sonicator (**2x** 00:00:30). Note: adjust times and intensity according to the available instrument. 30s

10 Clear lysate by spinning in a Beckman centrifuge, at 40000 x g, 4°C, 00:30:00 , Ti45 Rotor . 30m

11 Inject soluble fraction onto a 5ml HT column operating at 4 °C pre-equilibrated in Buffer A at 1ml/min flow rate.

12 Wash column for 5 µL with Buffer A at 2 ml/min flow rate to remove unspecific bound proteins.

13 Elute protein at 300mM Imidazole concentration. Perform elution at 1ml/min flow rate.

14 Perform TEV cleavage with 1mg/ml TEV protease overnight at 4˚C while dialyzing against elution buffer containing no imidazole.

15 Apply cleavage reaction to equilibrated HT column, and collect the flow-through.

16 Measure protein absorbance at A_{280} with a Spectrophotometer against dialysis buffer.

17 Aliquot protein, flash freeze in liquid Nitrogen and store at -80 °C .