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

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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.

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KEYWORDS

expression, purification, recombinant, Microtubule-associated proteins 1A/1B light chain 3B, LC3B, mCherry-tag

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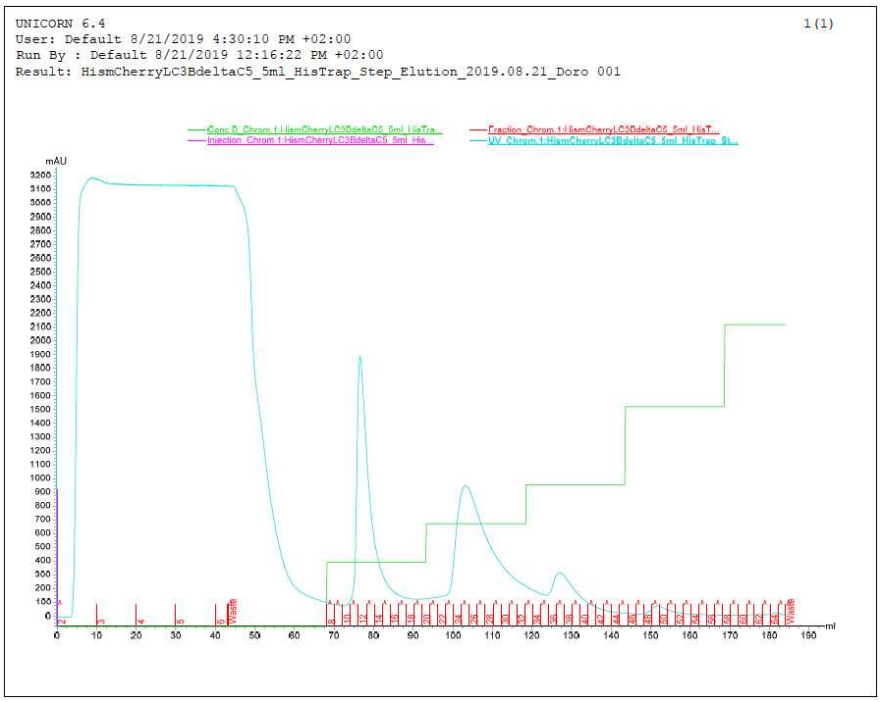
PROTOCOL INTEGER ID

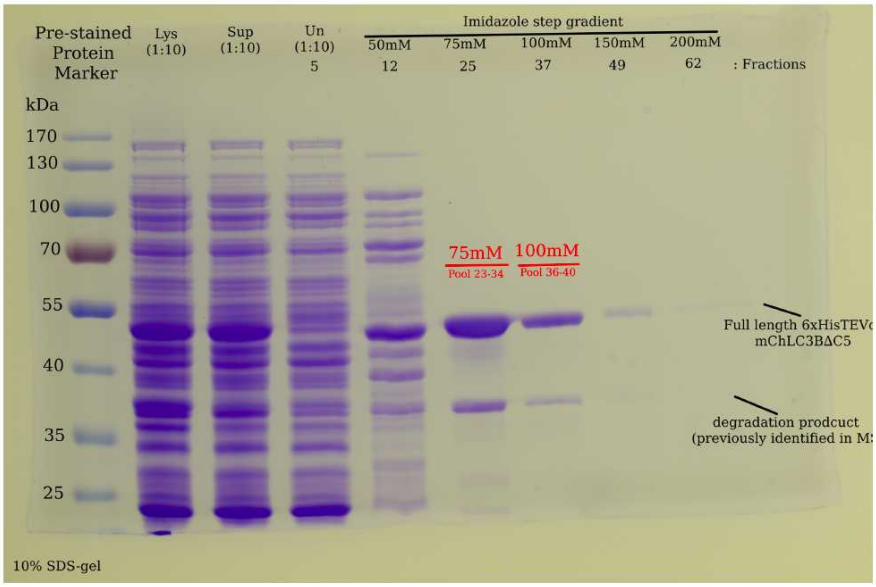
48436

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

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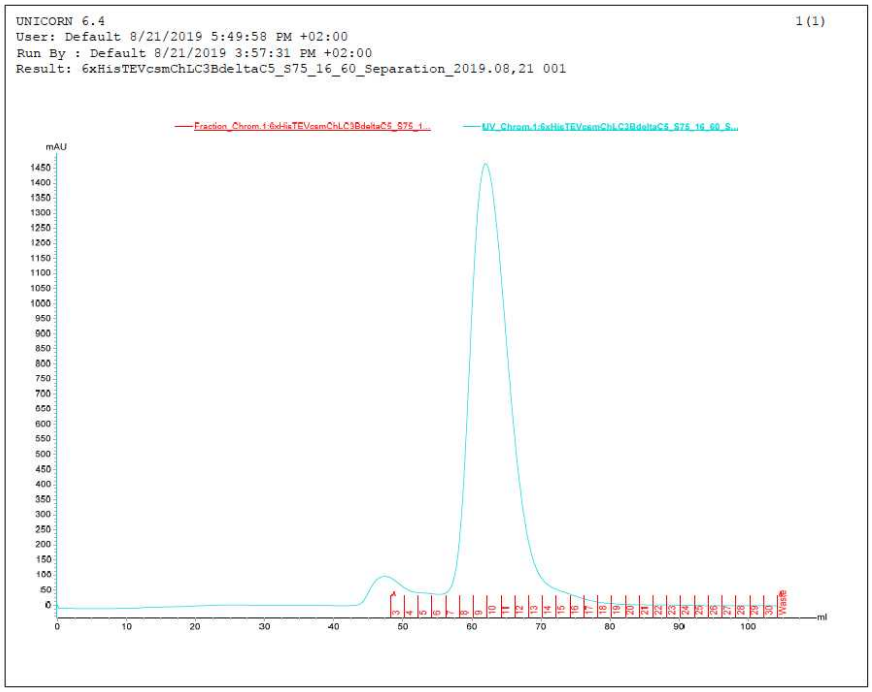
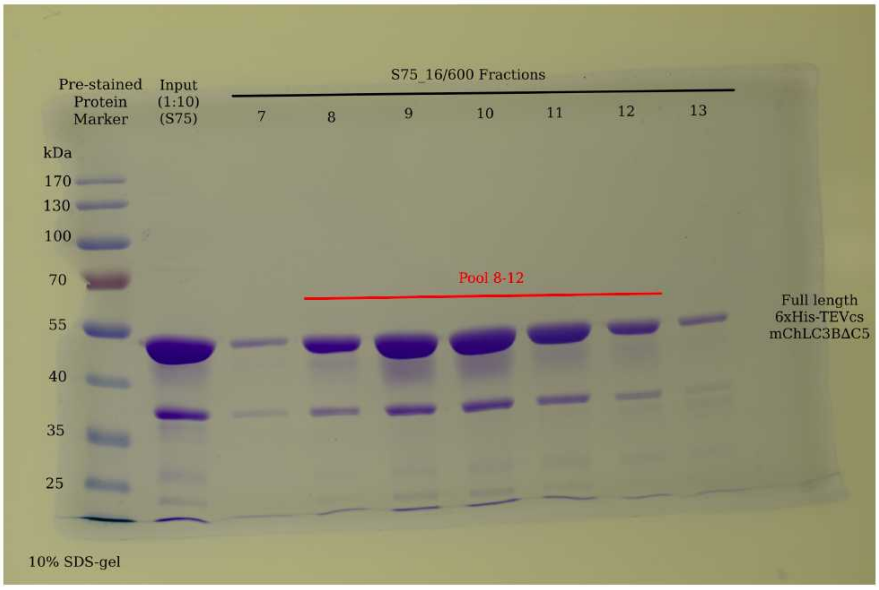


Chromatograph of Size Exclusion purification for mCherry-hLC3B.



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

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MATERIALS TEXT

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 STARTING

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 169168 , SMC948) into E.Coli Rosetta pLyss cells and plate on Ampicillin/Chloramphenicol LB agar plate for **Overnight** at **37 °C** .

2

The following day, inoculate a **100 mL LB + Amp/Cam pre-culture** with 1-2 colonies and grow **Overnight** at **37 °C** shaking.

3 The following day, use **20 mL pre-culture** to inoculate **1 L LB medium + Amp/Cam** at **37 °C** until an OD_{600} (Optical Density) of 0.4 is reached.

4 Cool down the culture to **18 °C** and grow to an OD_{600} = 0.8.

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5 Induce protein expression with [M]**100 Micromolar (µM) IPTG** and grow for a further **16:00:00** at **18 °C** 16h shaking.

6 15m

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).

7

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

Protein Purification

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

Purification of LC3B Ubiquitin-like modifier 30m 30s

9 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** .

10 30s

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

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

12 Filter supernatant through a 0.45 µm filter and inject onto a 5ml HT column operating at **4 °C** pre-equilibrated in Buffer A at 1ml/min flow rate.

13

Wash column for **5 column volumes (CV) with Buffer A** at 2 ml/min flow rate to remove unspecific bound proteins.

14 Elute protein through a step elution gradient in 50mM, 75mM, 100mM, 150mM, 200mM and 300mM Imidazole concentration. Perform elution at 1ml/min flow rate. Collect peak fractions at each step.

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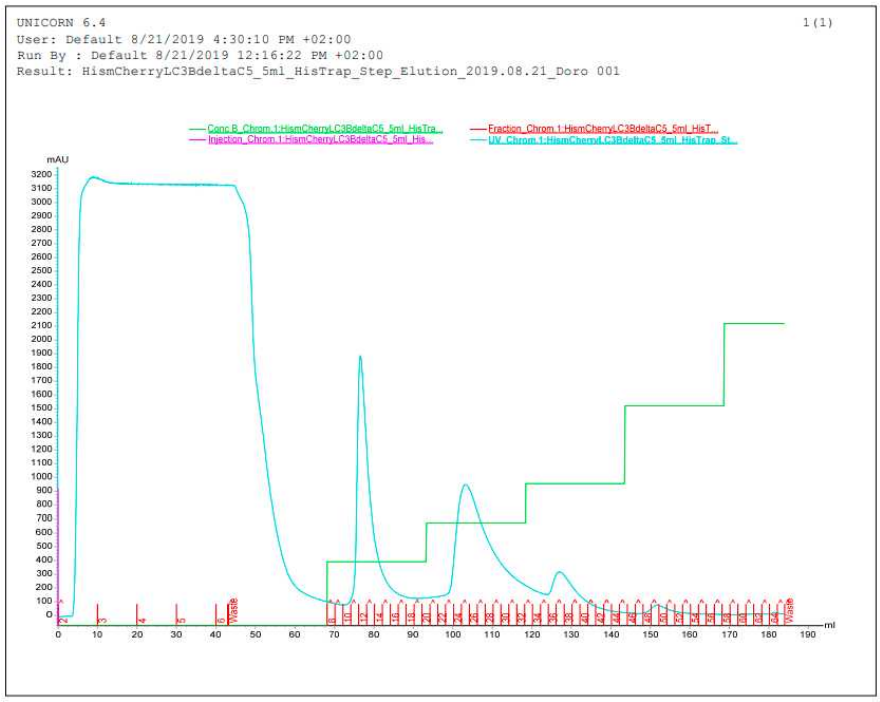
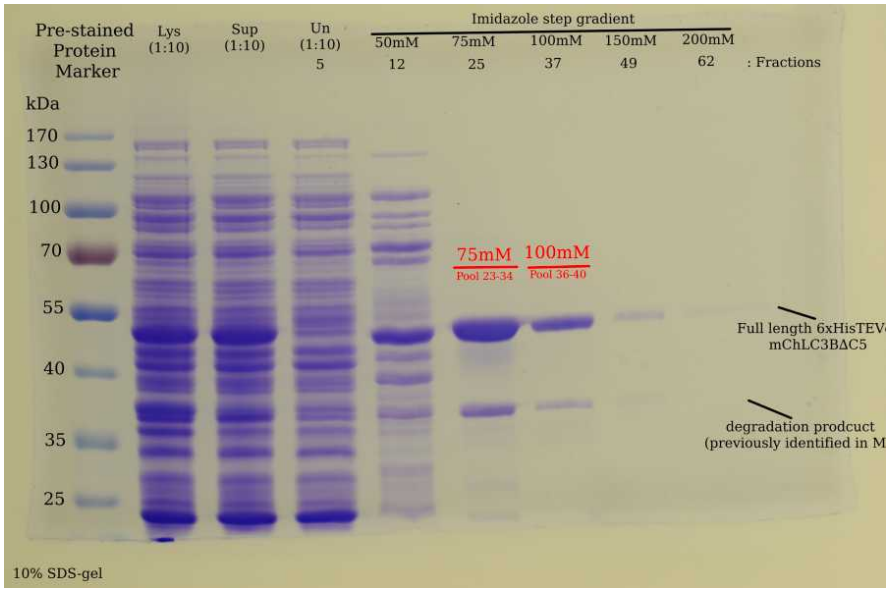


Chromatograph of His-tag affinity purification for mCherry-LC3B.

15 Check peak fractions of each step on a SDS-PAGE (see gel below). Pool and concentrate those containing the protein of interest (75 and 100mM Imidazole step) by spinning at **4 °C** down in an Amicon Filter 30kDa cut-off to **2 mL final volume** .

Centrifugation steps are kept short (**00:05:00**) to avoid protein local concentration/aggregation on the filter.



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

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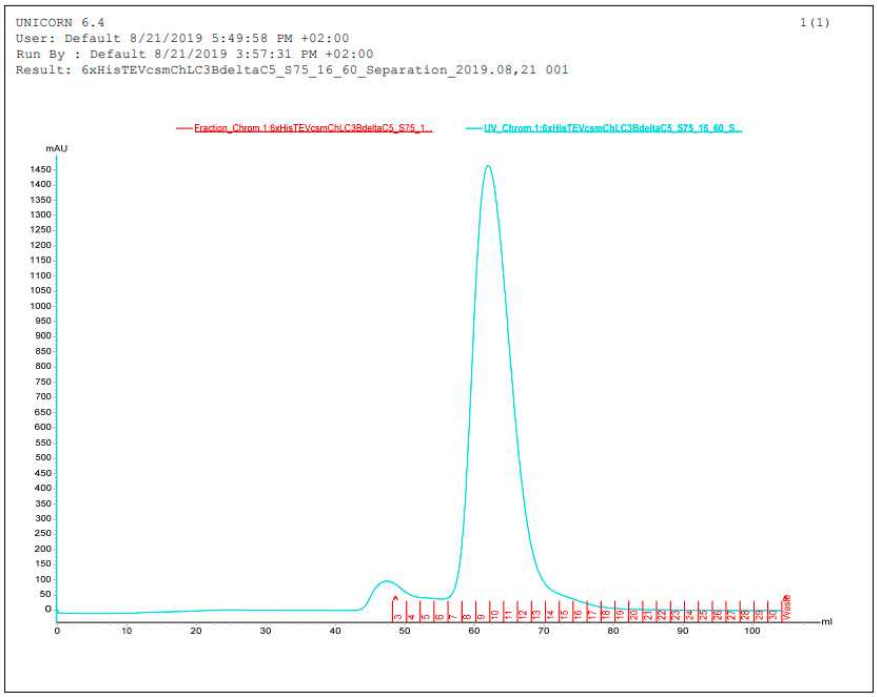
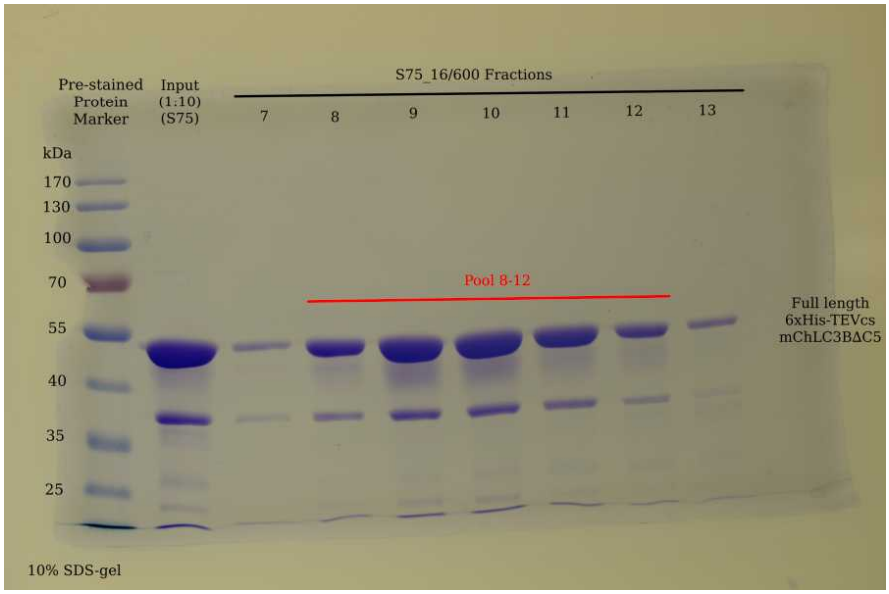
16 Inject **2 mL protein** onto a S75_16/60 column pre-equilibrated in SEC buffer.



Chromatograph of Size Exclusion purification for mCherry-hLC3B.

17 Check fractions on a 10% SDS-PAGE (see gel below). Pool and concentrate those containing the proteins of interest down at **4 °C** in an Amicon Filter 30kDa cut-off.

Centrifugation steps are kept short (**00:05:00**) to avoid protein local concentration/aggregation on the filter.



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

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18

Measure protein absorbance at A_{280} with a Spectrophotometer against Size Exclusion Chromatography purification buffer (MW = 47,89 kDa ; Extinction coefficient = 41830 $M^{-1}cm^{-1}$).

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

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