DEC 02, 2022

WORKS FOR ME 1

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

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1

Dorotea Fracchiolla[1], Liv Jensen[2]

[1]Team Hurley; [2]University of California, Berkeley

Liv Jensen

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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.

DOI

dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1

PROTOCOL CITATION

Dorotea Fracchiolla, Liv Jensen 2022. Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

https://doi.org/10.1083/jcb.201912098

FORK NOTE

FORK FROM

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

KEYWORDS

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

CREATED

Dec 02, 2022

LAST MODIFIED

Dec 02, 2022

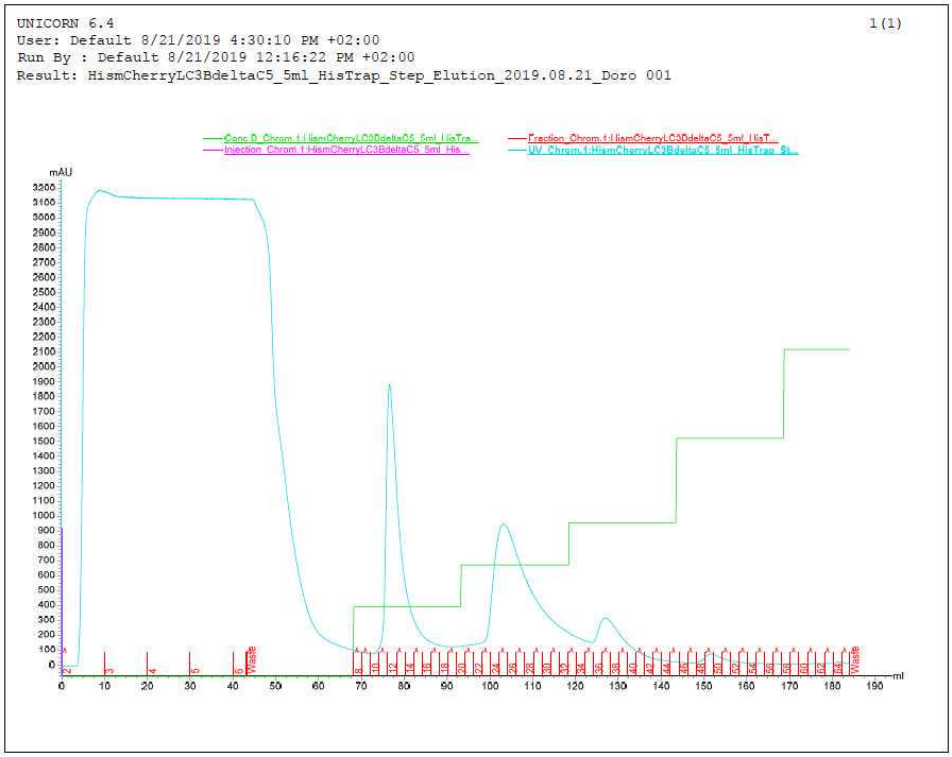
PROTOCOL INTEGER ID

73497

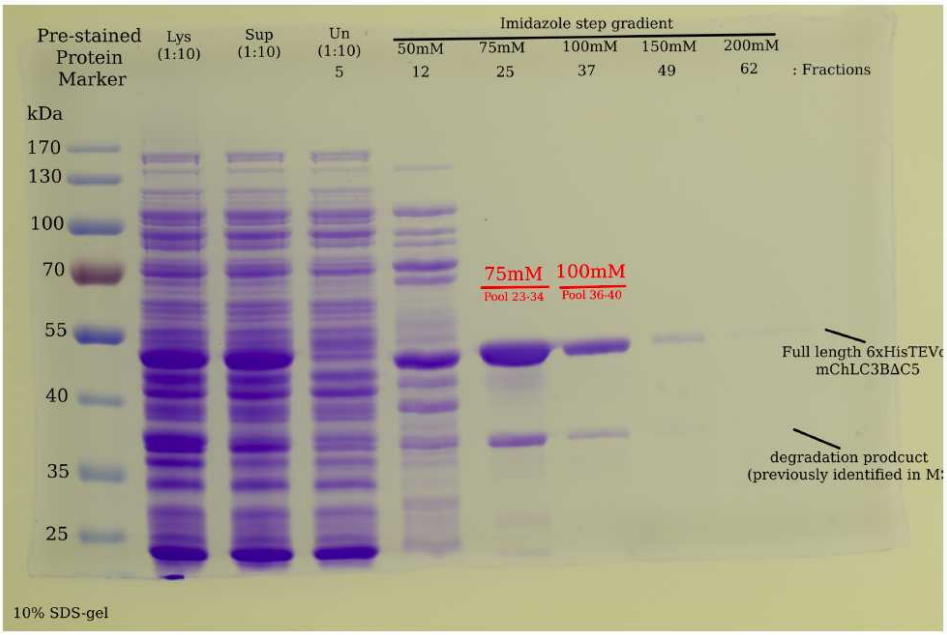
GUIDELINES

Affinity purification

Citation: Dorotea Fracchiolla, Liv Jensen Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier https://dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1



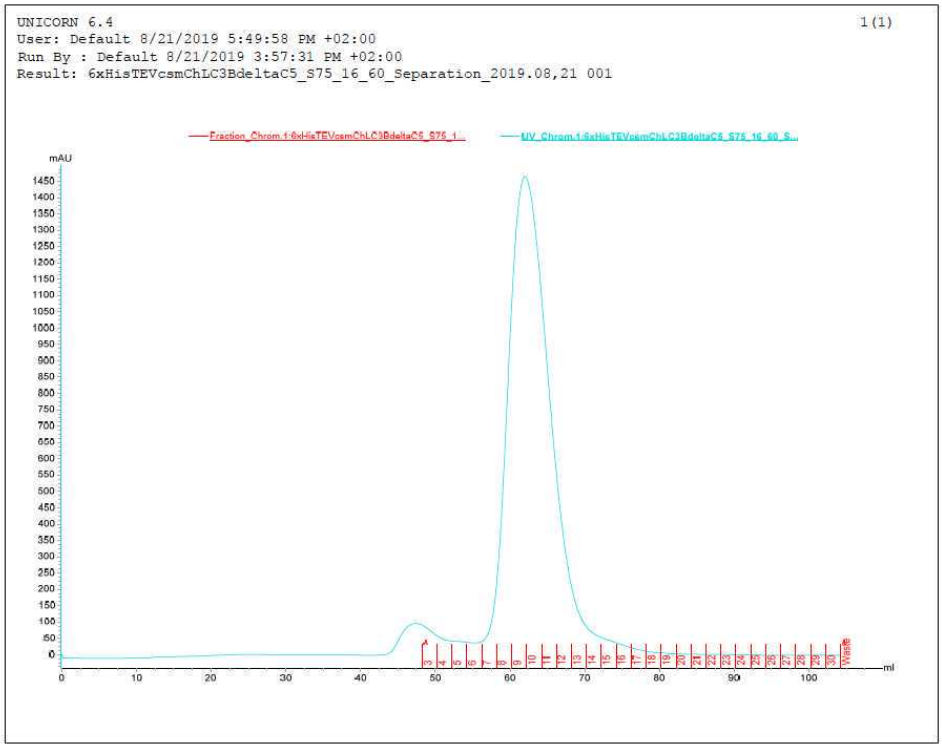
Chromatograph of His-tag affinity purification for mCherry-hLC3B.



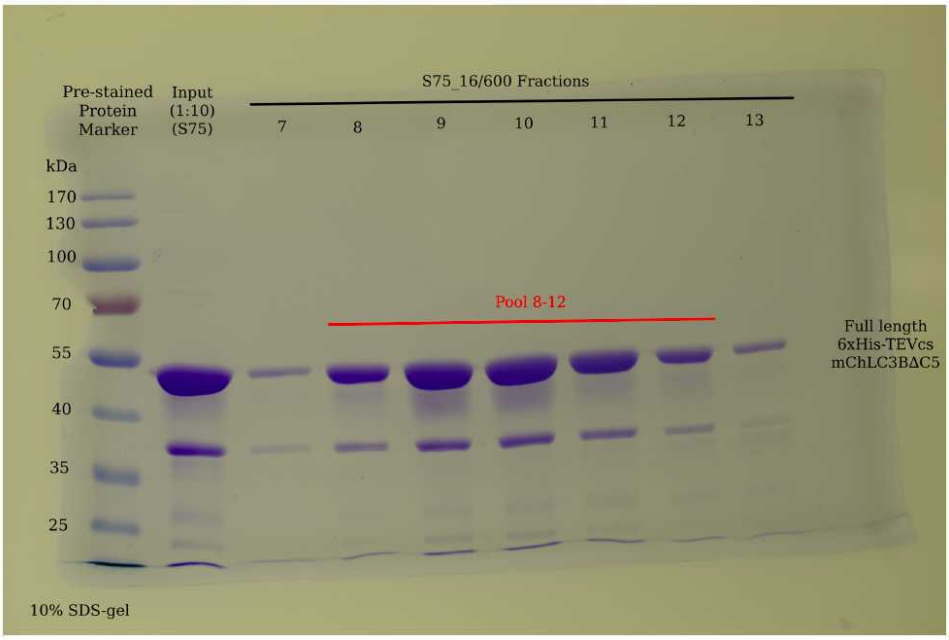
Coomassie BB stained gel of His-tag affinity purification for mCherry-hLC3B.

Size Exclusion Chromatography

Citation: Dorotea Fracchiolla, Liv Jensen Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier https://dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1



Chromatograph of Size Exclusion purification for mCherry-hLC3B.



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

Citation: Dorotea Fracchiolla, Liv Jensen Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier https://dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1

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.

DISCLAIMER

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.

Citation: Dorotea Fracchiolla, Liv Jensen Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier https://dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1

16h 15m

Protein Expression

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.

16h

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

15m

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).

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.

30m 30s

Purification of LC3B Ubiquitin-like modifier

8 Cells are lised via freeze/thaw cycles and sonication: thaw pellet corresponding to 1 L culture by

Citation: Dorotea Fracchiolla, Liv Jensen Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier https://dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1

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 undetermined 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.

Citation: Dorotea Fracchiolla, Liv Jensen Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier https://dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1

Citation: Dorotea Fracchiolla, Liv Jensen Expression and purification protocol of the Human (Homo sapiens) LC3B Ubiquitin-like modifier https://dx.doi.org/10.17504/protocols.io.j8nlkw82dl5r/v1