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Purification of recombinant Low Density Lipoprotein Receptor Related Protein Associated Protein 1 (LRPAP1, RAP) from Escherichia coli

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ABSTRACT

This protocol details how to efficiently purify LDL Receptor Related Protein Associated Protein 1 (LRPAP1 or RAP) from Escherichia coli.

ATTACHMENTS

Purification of recombinant LRPAP1 from E. coli.docx

Protocol status: Working
We use this protocol and it's working

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MATERIALS

Buffers

- **Lysis buffer:**

	A	B
	Tris-Cl pH 8.0	50 mM
	NaCl	300 mM
	Imidazole	10 mM

- **High salt buffer:**

	A	B
	Tris-Cl pH 8.0	50 mM
	NaCl	300 mM
	Imidazole	250 mM

- **Low salt buffer:**

	A	B
	Tris-Cl pH 8.0	50 mM
	NaCl	10 mM

- Size exclusion chromatography (SEC) buffer: 1x Phosphate buffered saline (PBS) pH 7.4

pQTEV-LRPAP1 **addgene Catalog #31327**

LRPAP1 express4ion

18h 37m 45s

1 Thaw RbCl-competent Escherichia coli Bl21 cells (DE3) On ice.

2 Add 1 µL of pQTEV-LRPAP1 plasmid without the signal peptide (1-35 amino acids) and incubate 00:30:00 On ice. 30m

3 Heat shock 00:00:45 at 42 °C. 45s

4 Incubate On ice 00:02:00, then add 850 µL Lysogeny broth (LB) or Super Optimal broth with Catabolite repression (SOC) medium. 2m

5 Shake for 01:00:00 at 37 °C. 1h

6 Centrifuge for 3000 x g, 00:05:00 and remove most of the supernatant. 5m

7 Resuspend the pellet with the remaining supernatant and plate the bacteria on LB /Ampicillin agar plates and incubate Overnight at 37 °C. 8h

8 Prepare preculture: Scrap all colonies with the scraper and inoculate 25-50 mL LB/Ampicillin. Shake at 37 °C for 4-6 h.

9 Measure OD_{600} of the preculture and inoculate 6 L of LB media to an OD_{600} = 0.05.

10 Shake flasks at 37 °C until approx. OD_{600} = 0.5-0.8. (2-4 h).

11 Add isopropyl β-D-1-thiogalactopyranoside (IPTG) at final concentration of [M] 1 millimolar (mM).

12 Shake flasks Overnight at 22 °C. 8h

13 Centrifuge bacterial culture at 4000 rpm, 01:00:00. Discard supernatant. 1h

14 Resuspend each pellet with Lysis buffer (20 mL/1L bacteria) supplemented with Complete EDTA-free protease inhibitor cocktail (Merck). Flash-freeze in liquid nitrogen for storage at -80 °C.

Lysis 1h 15m 50s

15 Thaw the cell pellets in a water bath at 22 °C and add lysis buffer (final volume 200 mL lysis buffer/ 6L bacteria) supplemented with Complete EDTA-free protease inhibitor cocktail (Merck) and Sm DNase 50 undetermined.

16 Add 1 undetermined lysozyme and incubate gently shaking for 00:30:00 at 4 °C. 30m

17 Sonicate lysate On ice, 8 cycles 00:00:20 ON, 00:00:30 OFF. 50s

18 Centrifuge lysate at 40000 rpm, 4°C, 00:45:00. 45m

Ni-NTA chromatography

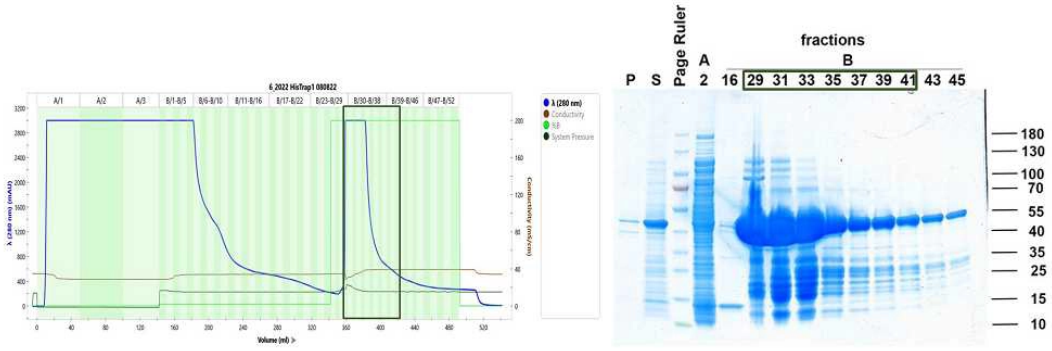
19 Equilibrate the Ni-NTA column with 10 column volumes (CV, 20 mL) Lysis buffer.

20 Load lysate supernatant to the Ni-NTA column.

21 Wash the Ni-NTA column with 10 CV Lysis buffer.

22 Elute His7-TEV-RAP with 5 CV 100% High salt buffer and collect elution fractions.

23 Analyze eluted fraction by SDS-PAGE and Coomassie blue staining.



Ni-NTA chromatogram and SDS PAGE analysis. P: 20 µL resuspended pellet + 20 µL 2x SDS sample buffer, loaded 15 µL. S: 20 µL diluted supernatant + 20 µL 2x SDS sample buffer, loaded 15 µL. Fractions of interest: 10 µL + 10 µL 2x SDS sample buffer; loaded 6 µL. Green box: Collected elution fractions (Fractions 29-41, 65mL).

Desalting

24 In order to reduce the salt concentration, load the eluted protein onto a HiPrep 26/10 desalting column equilibrated with the Low salt buffer.

His-TEV cleavage

8h

25 Collect eluted fraction containing protein and add glycerol at final concentration of 10%, DTT at final concentration of [M] 1 millimolar (mM), EDTA at final concentration of [M] 0.25 millimolar (mM) and His-TEV at final concentration of 93U per mg of protein.

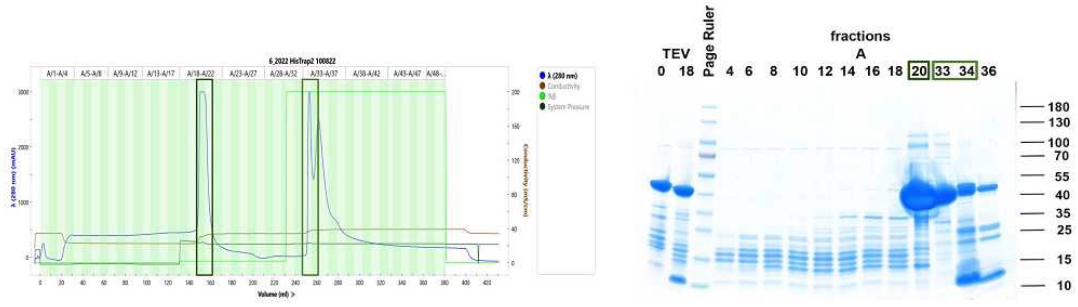
26 Incubate at 4 °C Overnight . 8h

Ni-NTA chromatography (Collect flow through)

27 Load the cleavage mixture onto a Ni-NTA column previously equilibrated with Lysis buffer and collect the flow through where the cleaved RAP protein should elute.

28 Wash the column with 5 CV (CV, 20 mL) of Lysis buffer and collect eluted fractions.

29 Analyze flow though fractions by SDS-PAGE and Coomassie blue staining.



Ni-NTA chromatogram and SDS PAGE analysis. TEV digest: 10 µL sample + 10 µL 2x SDS sample buffer, loaded 2.0 µL. Fractions of interest: 10 µL sample + 10 µL 2x SDS sample buffer, loaded 5 µL. Green boxes: Collected flow through (Fractions 20-21, 16 mL), and uncleaved His7-TEV-RAP protein eluted from the Ni-NTA column (Fractions 33-34).

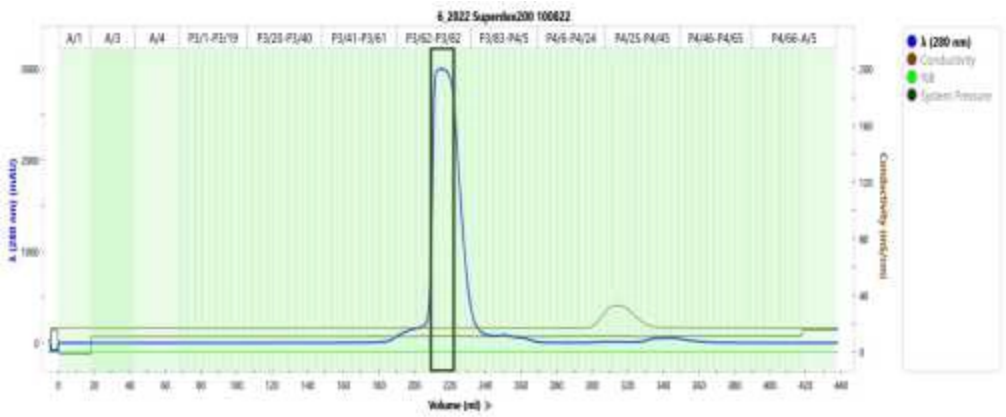
Note

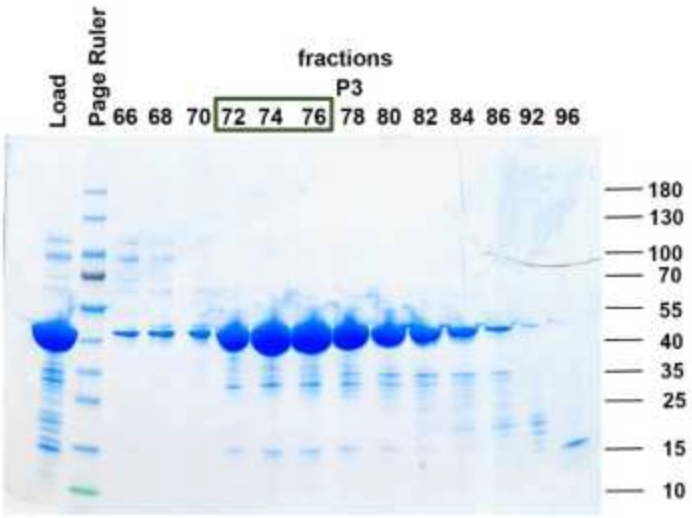
Some uncleaved His7-TEV-RAP protein may be eluted from the Ni-NTA column. Those fractions can be pooled, desalted and TEV digested again.

Size exclusion chromatography

30 Load RAP-containing fractions onto a Superdex-200 column previously equilibrated with SEC buffer.

31 Analyze eluted fractions by SDS-PAGE and Coomassie blue staining.





Size exclusion chromatogram and SDS PAGE analysis. 10 µL fraction of interest + 10 µL 2x SDS sample dye, loaded 2.0 µL. Green box: Collected eluted fractions (72-77, 12mL).

32 Pool fractions containing RAP aliquot and flash-freeze in liquid nitrogen for storage at -80 °C.

Note

The protein can be concentrated with a filter device like a VivaSpin20, MWCO 10,000 Da. **Approximate yield**: from 6 L of bacterial culture around 95 mg of pure RAP are obtained.