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GFP-Clu-tails purification from Escherichia coli cells

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We use this protocol and it's working

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Abstract

This protocol details how to purify the fusion protein GFP-Clu-tails from Escherichia coli.

Attachments

DOCX

GFP-Clu-tails purifi...

1.7MB

Materials

Buffers

Binding buffer: PBS + [M] 20 millimolar (mM) imidazole

Elution buffer: PBS + [M] 250 millimolar (mM) imidazole

His_6-Ubiquitin-GFP-Clu-tails expression and cell lysis

1 Express His_6-Ubiquitin-GFP-Clu-tails in E. coli Bl21 (DE3) codon+RIL cells cultured in 4 L LB Medium Overnight at 18 °C with [M] 0.25 millimolar (mM) IPTG.

2 Centrifuge culture and keep pellet.

3 Re-suspend pellet in 70 mL volume of ice-chilled Binding buffer, add Complete protease inhibitor cocktail (Roche) and [M] 1 millimolar (mM) phenylmethylsulfonyl fluoride (PMSF).

4 Lyse cells by ultrasonication in ice bath (15 cycles of 00:00:25 ultrasonication with 00:01:35 intermittent cooling). 2m

5 Clear lysate by centrifugation at 22000 rpm in a JA25.50 rotor at 4 °C .

Ni2+-chelating Sepharose metal affinity chromatography

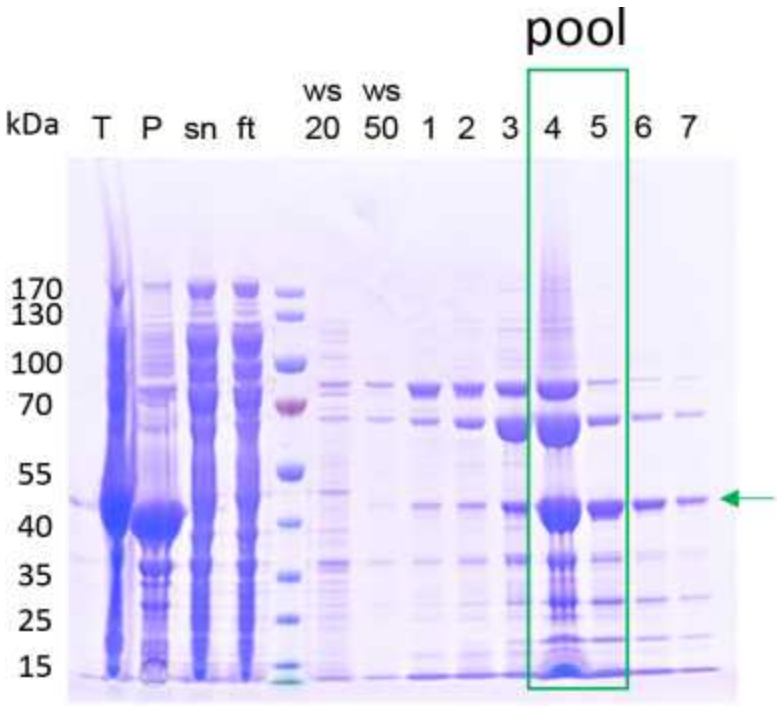
6 Load supernatant onto a 8 mL Ni-chelating Sepharose (Cytiva 17-0575-01) column previously equilibrated with binding buffer by gravity flow at 4 °C .

7 Wash the column with 5 CV of ice-chilled Binding buffer.

8 Wash the column with 2 CV of ice-chilled PBS + [M] 50 Molarity (M) imdiazole.

9 Elute His6-Ubiquitin-GFP-Clu-tails protein with 6x 5 mL of ice-chilled Elution buffer. Collect fractions of 5 mL volume. Bright green color indicates presence of His6-Ubiquitin-GFP-Clu-tails. Store fractions On ice .

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



Protease cleavage and removal of protease

11 Pool fractions containing His6-Ubiquitin-GFP-Clu-tails peak (here fractions 4 and 5). Cleave off His6-Ubiquitin with His-tagged Usp2 during 04:00:00 On ice . 4h

12 Exchange protein buffer to Binding buffer with a HiPrep™ 26/10 Desalting column (Cytiva 17-5087-01) equilibrated with Binding buffer at 4 °C using bright green fluorescence as a marker for protein containing fractions.

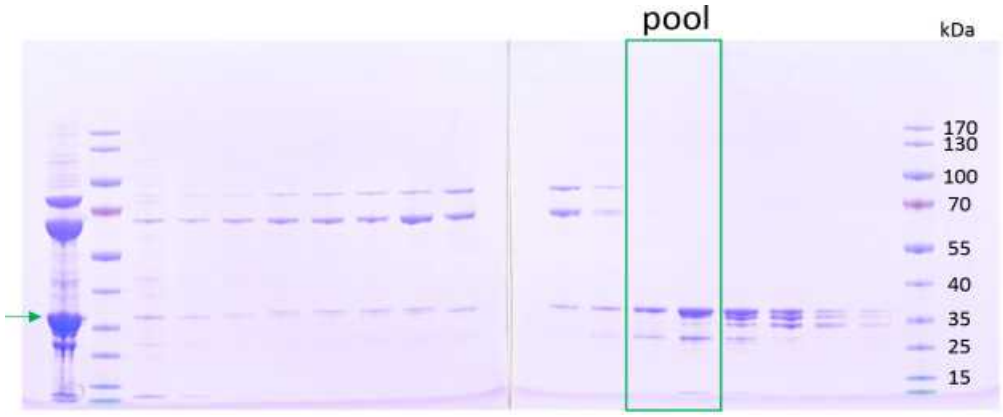
13 Remove uncleaved material and His-tagged Usp2 by metal affinity chromatography as above. Collect flow-through and wash fractions with bright green fluorescence.

14 Concentrate to 1.5 mL volume by ultrafiltration using 10 kDa cut-off spin concentrator at 4 °C .

Size exclusion chromatography on HiLoad 16/600 Superdex-200 (Cytiva 28-9893-35)

15 Apply concentrate on a HiLoad 16/600 Superdex-200 (Cytiva 28-9893-35) column equilibrated with PBS. Develop the column at 4 °C and collect 3 mL fractions.

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



17 Merge fractions 16 and 17. Concentrate to 0.7 mL volume by ultrafiltration using 10 kDa cut-off spin concentrator at 4 °C, aliquot and flash-freeze purified GFP-Clu-tails in liquid nitrogen for storage at -70 °C.

Note

Concentrations were determined by absorbance at 488 nm (eGFP!) using absorbance coefficients of 61,000 M^{-1} cm^{-1} or 1.45 L g^{-1} cm^{-1}.
Approximate yield: From 4 L of culture around 2.5 mg of GFP-Clu-tails were obtained.