MBP-Clu-tail purification from Escherichia coli cells

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Abstract

This protocol details how to efficiently purify the fusion protein Maltose binding protein (MBP)-Clu-tail (204-238) from Escherichia coli.

Attachments

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MBP-Clu(204-238) pur...

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Materials

Buffers

- **Binding buffer:**

	A	B
	Tris/HCl pH 7.4	20 mM
	NaCl	200 mM
	EDTA	1 mM

- Elution buffer: Binding buffer + [M] 10 millimolar (mM) maltose (final concentration)

Troubleshooting

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

1 Express MBP-Clu-tail in E. coli BI21 (DE3) codon+RIL cells cultured in 1 L LB Medium containing 2 µL glucose at 37 °C with [M] 1 millimolar (mM) IPTG during 02:00:00 . 2h

2 Centrifuge culture and keep pellet.

3 Re-suspend pellet in 25 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 (10 cycles of 00:00:30 ultrasonication with 00:01:30 intermittent cooling). 2m

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

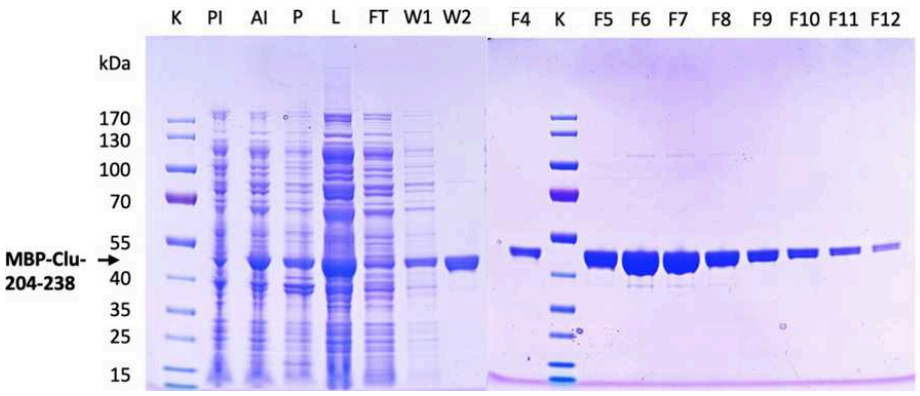
Amylose affinity chromatography

6 Load supernatant onto a 20 mL Amylose Resin (New England Bioloabs) column previously equilibrated with binding buffer by gravity flow at 4 °C .

7 Wash the column with 12 CV of ice-chilled binding buffer.

8 Elute MBP-Clu-tail protein with 12× 3 mL of ice-chilled Elution buffer. Collect fractions of 3 mL volume. Store fractions On ice .

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



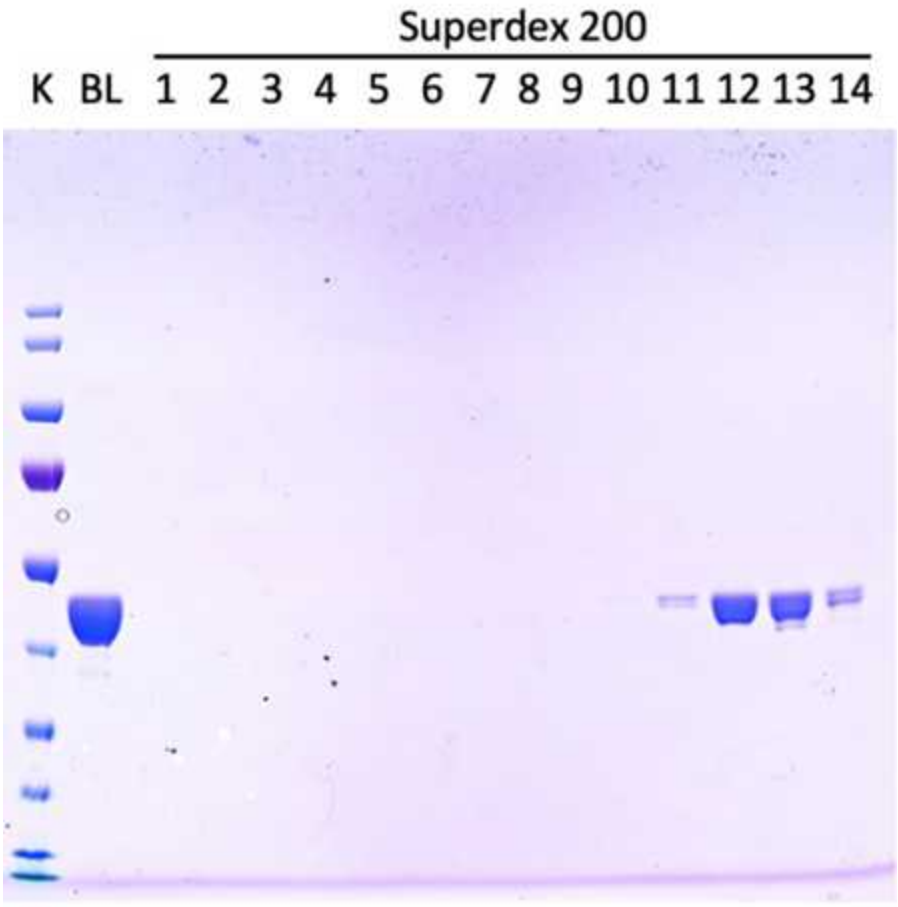
10 Pool fractions containing MBP-Clu-tail.

11 Concentrate pool to less than 10 mL volume by ultrafiltration using 10 kDa cut-off spin concentrator at 4 °C.

Size exclusion chromatography

12 Apply concentrate on a HiLoad 26/600 Superdex-200 (Cytiva 28-9893-36) column equilibrated with PBS. Develop the column at 4 °C and collect 10 mL fractions.

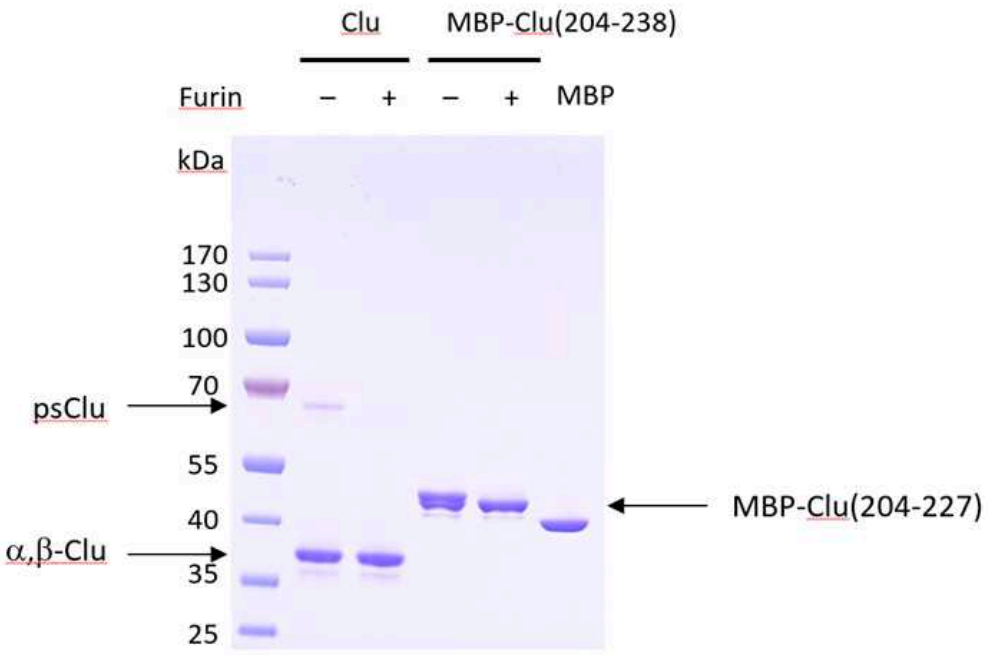
13 Analyze eluted fractions by SDS-PAGE and Coomassie blue staining.



14 Merge fractions with MBP-Clu-tail peak. Concentrate to 1.5 mL volume by ultrafiltration using 10 kDa cut-off spin concentrator at 4 °C, aliquot and flash-freeze purified MBP-Clu-tail in liquid nitrogen for storage at -70 °C.

Note

MBP-Clu-tail appears as a double band. In contrast to the lower band, the upper band is sensitive to cleavage by furin, suggesting that a protease from E. coli partially cleaves close to the furin site in MBP-Clu-tail.



Note

Concentrations were determined by absorbance at 280 nm using absorbance coefficients of 66,350 M^{-1} cm^{-1} or 1.645 L g^{-1} cm^{-1} for MBP-Clu-tail.

Approximate yield: From 1 L of culture around 15 mg of pure MBP-Clu-tail were obtained.