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Purification of CHMP2B

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We use this protocol and it's working

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Abstract

This protocol describes a method for purification of recombinant CHMP2B from E Coli. To improve solubility, CHMP2B is tagged with His-SUMO and expressed at a low temperature. The His-tagged SUMO fusion is first isolated on a nickel column before cleavage of the SUMO tag and a reverse nickel column to separate His-SUMO from the cleaved CHMP2B. The CHMP2B is then cleaned up by size exclusion.

Materials

- BL21 (DE3) E Coli
- LB media (VWR cat. no. J106-2 kg) LB Broth **Amresco Catalog #**J106-2KG
- Terrific Broth media (Sigma cat. no. T0918)

Terrific Broth, modified **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T0918

- Kanamycin sulfate (Sigma cat. no. K4000-50G)

Kanamycin sulfate from Streptomyces kanamyceticus **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**K4000-50G

- IPTG (1M stock in water) (Roth cat. no. CN08.3) IPTG, 50 g **Carl Roth Catalog #**CN08.3
- pET28-His-SUMO-CHMP2B plasmid
- Incubator capable of 18 °C and 37 °C
- Tip sonicator
- Nanodrop or other spectrophotometer capable of measuring 220 nm absorbance
- FPLC
- PBS (Thermo Fisher Scientific ca. no. 20012068) PBS, pH 7.2 **Thermo Fisher Catalog #**20012068
- Lysis Buffer:

	A	B
	Tris	50 mM
	pH	8
	NaCl	500 mM
	Imidazole	10 mM
	β-Mercaptoethanol (β-ME)	2 mM
	glycerol	5 % (v/v)
	cOmplete Protease Inhibitor Cocktail (Roche cat. no. 11873580001)	1x

cOmplete™ EDTA-free Protease Inhibitor Cocktail **Roche Catalog #**11873580001

Note

β-ME stock is 14.3 M.

- Nickel Buffer 1A:

	Tris	50 mM
	pH	8
	NaCl	500 mM
	β-ME	2 mM

- Nickel Buffer 1B:

	A	B
	Tris	50 mM
	pH	8
	NaCl	500 mM
	Imidazole	500 mM
	β-ME	2 mM

- Dialysis Buffer:

	A	B
	Tris	20 mM
	pH	7.5
	NaCl	150 mM
	Imidazole	4 mM
	β-ME	2 mM

- Nickel Buffer 2A: 20 mM Tris pH 7.0, 150 mM NaCl, 2 mM β-ME

	A	B
	Tris	20 mM
	pH	7
	NaCl	150 mM
	β-ME	2 mM

- Nickel Buffer 2B: 20 mM Tris pH 7.0, 150 mM NaCl, 500 mM Imidazole, 2 mM β-ME

	A	B
	Tris	20 mM
	pH	7
	NaCl	150 mM
	Imidazole	500 mM
	β-ME	2 mM

- SEC Buffer: 20 mM Tris pH 7.0, 150 mM NaCl, 2 mM β-ME

	A	B
	Tris	20 mM
	pH	7
	NaCl	150 mM
	β-ME	2 mM

- Glycerol
- His-SenP2 protease (50 U/µl, Max Planck Institute of Biochemistry Core Facility)
- Superdex S200 16/60 pg column (GE cat. no. 28989335)

GE Hiload 16/60 Superdex 200 Pg **GE Healthcare Catalog #**28989335

- 3 Ni-NTA HisTrap Columns (Cytiva cat. no. 17524802)

3 Ni-NTA HisTrap HP, 5 × 5 ml **Cytiva Life Sciences Catalog #**17524802

- Vivaspin® 20, 10 kDa MWCO concentration spin column (Sigma cat. no. GE28-9323-60)

Vivaspin® 20, 10 kDa MWCO Polyethersulfone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**GE28-9323-60

- PES Syringe Filter 0.22 µm (Merck Millipore cat. no. SLGP033RB)

Millex-GP Syringe Filter Unit, 0.22 µm, polyethersulfone, 33 mm, gamma sterilized **Merck Catalog #**SLGPR33RB

- PES Vacuum Filter 0.22 µm (Corning cat. no. 431097)

Corning® 500 mL Vacuum Filter/Storage Bottle System, 0.22 µm Pore 33.2cm² PES Membrane, Sterile, 12/ **Corning Catalog #**431097

- 3.5 kDa Dialysis Tubing (Spectrum cat. no. 132724)

Spectra/Por 3 Dialysis Tubing 3.5 kD 45mm 50ft **Repligen (Spectrum Labs) Catalog #**132724

Culture Growth

16h

1 Transform BL21 (DE3) cells with pET28-His-SUMO-CHMP2B plasmid and grow an Overnight culture in 50 mL LB media with [M] 0.05 mg/mL Kanamycin at 37 °C .

2 On the next day, dilute the culture 1:100 and grow cells in Terrific Broth supplemented with [M] 0.05 mg/mL Kanamycin at 30 °C until it reaches an OD_{600} of 0.3.

3 Reduce the incubation temperature to 18 °C .

4 After the culture grows to an OD_{600} of 0.6, induce protein expression [M] 0.5 millimolar (mM) IPTG for 16:00:00 . 16h

5 Harvest cells and wash the pellet once with PBS.

6 Freeze the pellets in liquid nitrogen and store at -80 °C .

Lysis

1h 22m

7 Thaw cells On ice and resuspend (by pipetting) in lysis buffer. Use 50 mL lysis buffer per 1 L of culture.

8 Lyse cells via sonication with a tip sonicator (00:20:00 , 00:00:20 on, 00:01:40 off). 22m

9 Centrifuge lysate at 40000 x g, 01:00:00 . 1h

Note

Carefully check to ensure that all of the solid material sedimented and supernatant is clear.

Nickel Column

10 Equilibrate 3 x HisTrap columns with 98% Nickel Buffer 1A with [M] 10 millimolar (mM) Imidazole (2% Nickel Buffer 1B).

11 Filter supernatant with the vacuum filter and apply to the column.

12 Wash with 5 column volumes of Nickel Buffer 1A with [M] 10 millimolar (mM) Imidazole (2% Nickel Buffer 1B).

13 Wash with 4 column volumes of Nickel Buffer 1A with [M] 50 millimolar (mM) Imidazole (10% Nickel Buffer 1B).

14 Elute with 5 column volumes of Nickel Buffer 1B ([M] 500 millimolar (mM) Imidazole).

15 Check fractions by gel and pool the fractions with His-SUMO-CHMP2B.

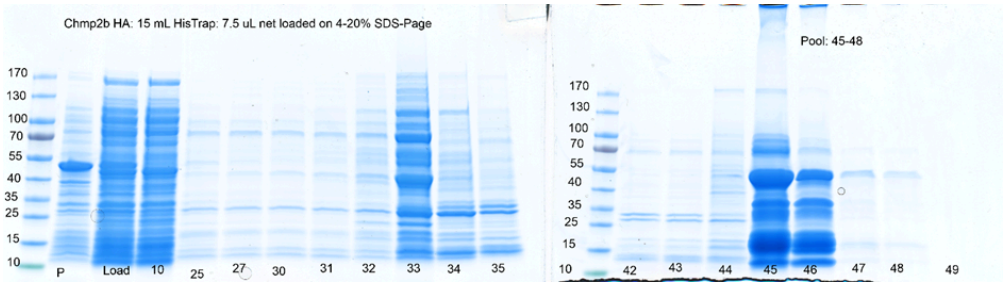

Figure 1. SDS-PAGE analysis of fractions (numbers below) before loading and after running the first nickel column. His-SUMO-CHMP2B runs around 50 kDa. In this purification, fractions 45-48 were pooled. P, pellet.

SUMO Tag Cleavage and Reverse Nickel Column

1m 40s

16 Add 150 µL His-SenP2 protease and glycerol to 10% final to the eluate, then dialyze into Dialysis Buffer Overnight .

1m 40s

17 Filter the sample through a 0.22 µL syringe filter.

18 Perform reverse Ni-NTA affinity purification with Nickel Buffer 2A and 2B, this time collecting the flow-through instead of the eluate.

Note

The now untagged CHMP2B will be in the flow-through.

19 Check the fractions by gel and pool the CHMP2B-containing fractions.

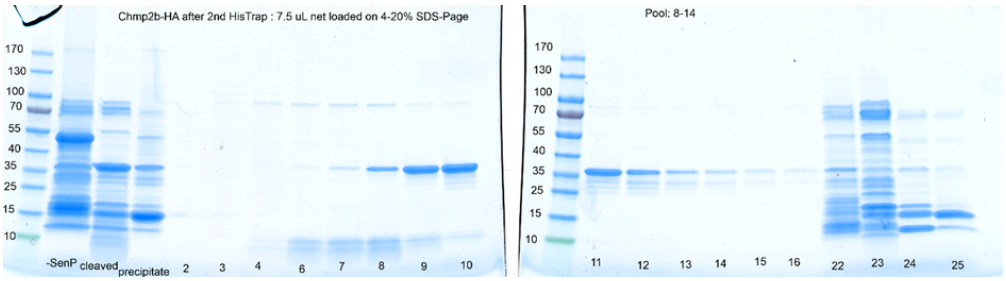

Figure 2. SDS-PAGE analysis of fractions (numbers below) before loading and after running the second nickel column. -SenP represents the sample before cleavage sith SenP2, the cleaved fraction represents what was loaded onto the column, and the precipitate comes from a 20000 rpm spin in a Ti 45 rotor, shown to demonstrate the propensity to aggregate after cleavage. CHMP2B runs around 35 kDa. In this purification, fractions 8-14 were pooled.

Size Exclusion

20 Concentrate the CHMP2B to a final volume of 5 mL with a concentration spin column.

21 Load the protein onto a Superdex S200 16/60 pg column. Collect 5 mL fractions for the first 30 mL of SEC buffer, then collect 1 mL fractions for the next 75 mL of SEC buffer.

a. CHMP2B will begin to elute around 70 mL.

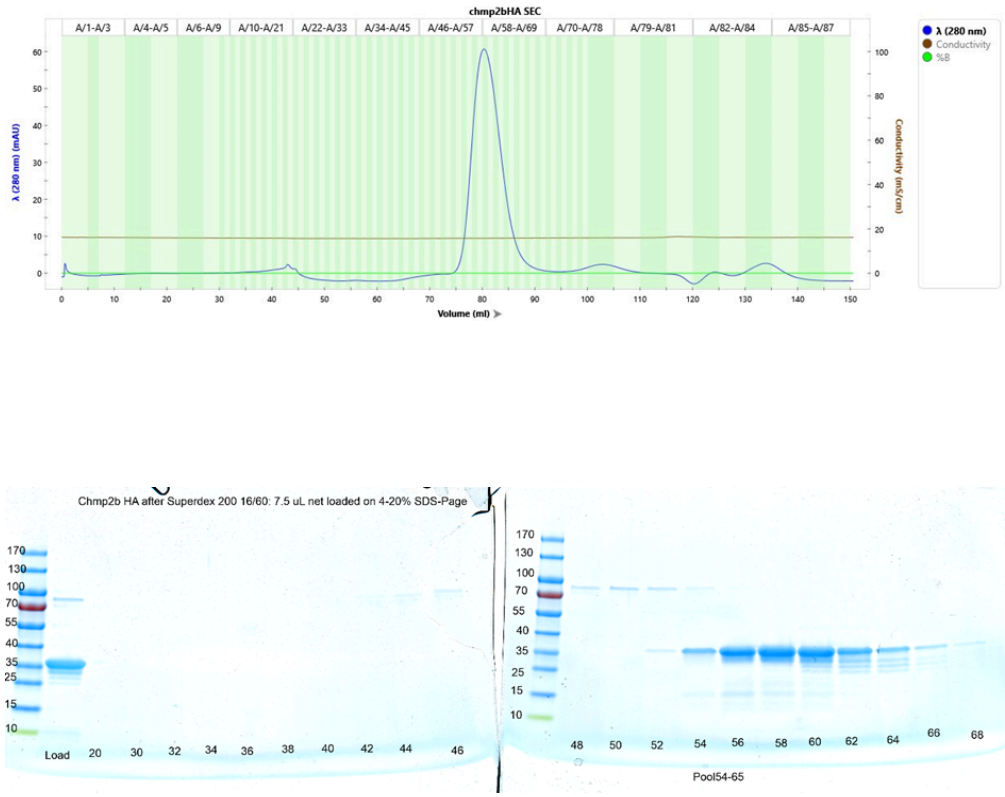

Figure 3. (Upper) Elution profile of cleaved CHMP2B on SEC. (Lower) SDS-PAGE gel of fractions before and after SEC. In this purification, fractions 54-60 were pooled.

22 Add 10% glycerol final to pooled fractions and concentrate the protein with a 10 kDa cutoff spin column to slightly more than 1.11 µL .

Note

a. CHMP2B has an extinction coefficient of 0. Concentration can be assessed Nanodrop at 220 undetermined or by a Bradford assay.
b. purified CHMP2B is now ready for aliquoting and snap freezing.