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Purification of Recombinant Human Hsc70 from *Escherichia coli*

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We use this protocol and it's working

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Abstract

This protocol details how to efficiently purify recombinant human heat shock cognate 71 kDa protein (Hsc70), also known as HSPA8, expressed as hexahistidine-tagged, TEV protease cleavable fusion protein in *Escherichia coli*.

Materials

Buffers:

- Lysis buffer:

	A	B
	HEPES-KOH pH 8.0	50 mM
	KCl	10 mM
	MgCl2	5 mM

- Elution buffer:

	A	B
	HEPES-KOH pH 8.0	50 mM
	KCl	10 mM
	MgCl2	5 mM
	Imidazole	1000 mM

- High salt buffer:

	A	B
	HEPES-KOH pH 8.0	50 mM
	KCl	1000 mM
	MgCl2	5 mM

- Size exclusion chromatography (SEC) buffer:

	A	B
	HEPES-KOH pH 7.5	50 mM
	KCl	150 mM
	MgCl2	5 mM

	A	B
	Glycerol	5%

Hsc70 Expression: Transformation

9h 37m 45s

1 Thaw RbCl-competent *Escherichia coli* BI21 cells (DE3) On ice .

2 Add 1 µL of pProEx-HtA Hsc70 plasmid 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 containing 100 µL Ampicillin agar plates and incubate Overnight at 37 °C . 8h

Hsc70 Expression: Bacterial Culture

18h 45m

8 **Prepare preculture:** Scrape all colonies with the scraper and inoculate 25 mL - 50 mL LB containing 100 µL Ampicillin in a flask. Shake flask at 37 °C for 04:00:00 - 06:00:00 . 6h

9 Measure OD_{600} of the preculture and inoculate 6 L of LB medium containing 100 µL Ampicillin (6 flasks) to an OD_{600} = 0.05.

10 Shake flasks at 37 °C until an approximate OD_{600} of 0.5-0.8 (02:00:00 - 04:00:00). 4h

11 Add isopropyl β-D-1-thiogalactopyranoside (IPTG) at final concentration of [M] 0.5 millimolar (mM) .

12 Shake flasks Overnight at 21 °C . 8h

13 Centrifuge bacterial culture at 4000 rpm, 00:45:00 in a JS-4.2 rotor (Beckman). Discard supernatant. 45m

14 Resuspend each pellet with Lysis buffer (20 mL per 1 L bacterial culture) supplemented with Complete EDTA-free protease inhibitor cocktail (Merck). Flash-freeze in liquid nitrogen for storage at -70 °C .

Hsc70 Purification: Lysis 1h 15m

15 Thaw the cell pellets in a water bath at 22 °C and add Lysis buffer (75 mL per 2 L bacteria) supplemented with Complete EDTA-free protease inhibitor cocktail (Merck), Sm DNase 50 U mL^{-1} and 1 µL lysozyme and incubate under gentle shaking for 00:30:00 at 4 °C . 30m

16 Lyse the cells with a Emulsiflex C5 high-pressure homogenizer (4 cycles at 20,000 psi).

17 Centrifuge lysate at 40000 rpm, 4°C, 00:45:00 . Keep clarified supernatant. 45m

Ni-NTA Chromatography

18 Equilibrate the Ni-NTA column (HisTrap HP column 5 mL; 17-5248-0, Cytiva) with 10 column volumes (CV, 15 mL) Lysis buffer.

19 Load lysate supernatant onto the Ni-NTA column (50 mL).

20 Wash the Ni-NTA column with 2 CV 99% Lysis buffer - 1% Elution buffer.

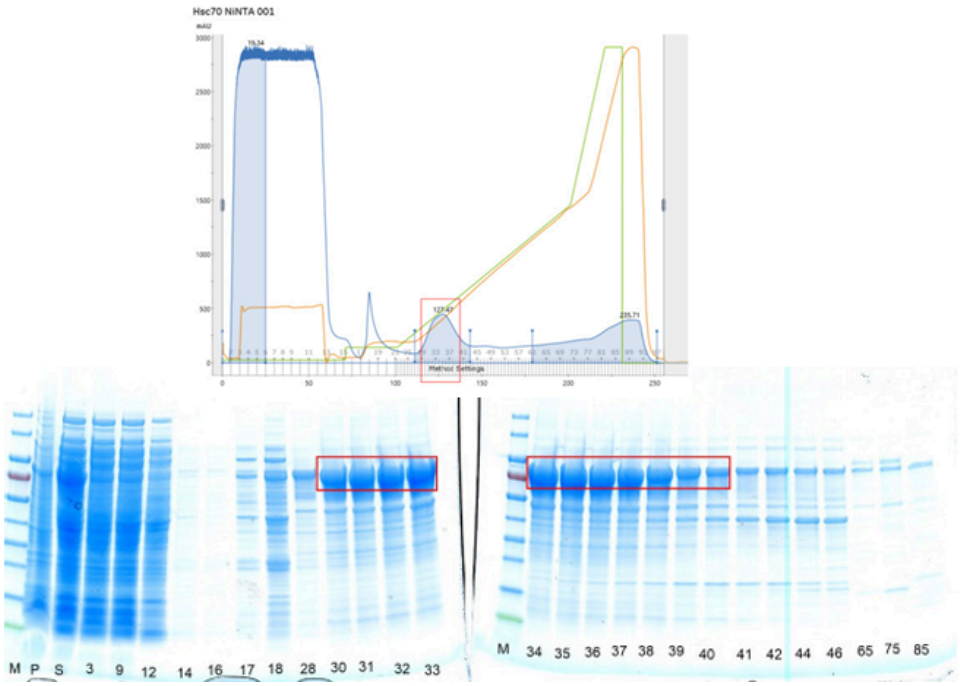
21 Wash the Ni-NTA column with 2 CV 95% Lysis buffer - 5% Elution buffer.

22 Elute His_6-TEV-Hsc70 with 10 CV gradient 5-50% Elution buffer and collect elution fractions.

23 Wash the Ni-NTA column with 2 CV gradient 50-100% Elution buffer.

24 Wash the Ni-NTA column with 1 CV gradient Elution buffer.

25 Analyze fractions by SDS-PAGE and Coomassie blue staining.



Ni-NTA chromatogram and SDS-PAGE analysis. P: 20 µL resuspended pellet + 20 µL 2x SDS PAGE sample buffer, loaded 15 µL. L: 20 µL lysate + 20 µL 2x SDS-PAGE sample buffer, loaded 15 µL. Fractions of interest: 10 µL + 10 µL 2x SDS-PAGE sample buffer; loaded 6 µL. Red boxes: Pooled elution fractions (Fractions 29-40, 30 mL).

Desalting

26 In order to exchange the buffer, load the eluted protein onto a HiPrep 26/10 desalting column (17-5087-01, Cytiva) equilibrated with 2 CV (1 CV, 55 mL) of Lysis buffer.

27 Load 15 mL of the collected elution fractions. Run several times if needed.

28 Wash the column with 2 CV of High salt buffer.

His-TEV Protease Cleavage

8h

29 Collect fractions eluted from desalting column containing protein and add His-TEV protease at final concentration of 400 U per mg of protein.

30 Incubate at 4 °C Overnight .

8h

Note

If precipitation is observed, reduce the salt concentration to [M] 150 millimolar (mM) KCl by dilution.

Ni-NTA Filter Column (Collect Flow Through)

31 Equilibrate the Ni-NTA column with Lysis buffer.

32 Load the cleavage mixture onto the column.

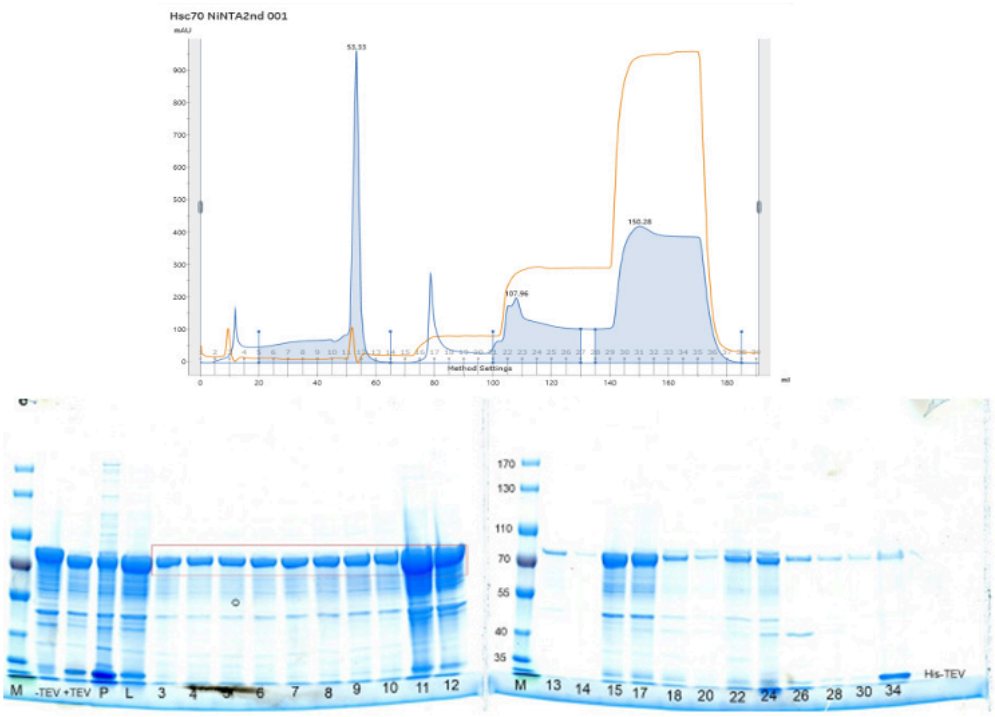
33 Collect the flow through where the cleaved Hsc70 protein should elute.

34 Wash the column with 3 CV 95% Lysis buffer - 5% Elution buffer.

35 Wash the column with 3 CV 75% Lysis buffer - 25% Elution buffer.

36 Wash the column with 3 CV Elution buffer and collect eluted fractions.

37 Analyze fractions of the flow-through by SDS-PAGE and Coomassie blue staining.



Ni-NTA chromatogram and SDS-PAGE analysis. -/+ TEV: 10 µL sample + 10 µL 2x SDS PAGE sample buffer, loaded 2.0 µL. Fractions of interest: 10 µL sample + 10 µL 2x SDS PAGE sample buffer, loaded 10 µL. Red box: Pooled flow-through fractions (Fractions 3-12, 55 mL).

Anion Exchange Chromatography

38 Equilibrate a MonoQ column (MonoQ HR10/10, 17-0506-01 Cytiva, CV: 8 mL) with 2 CV of Lysis buffer.

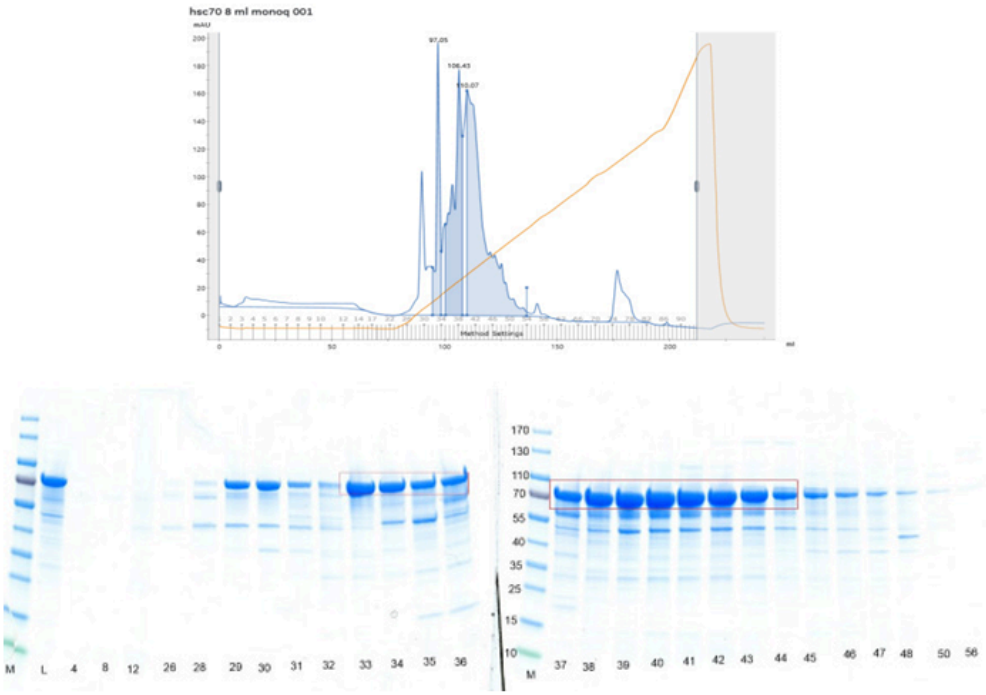
39 Load the pooled protein from the Ni-NTA column flow-through.

40 Wash with 2 CV of Lysis buffer.

41 Elute the protein with a gradient of 15 CV 1-70% High salt buffer.

42 Wash the column with a gradient of 2 CV 70-100% High salt buffer.

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



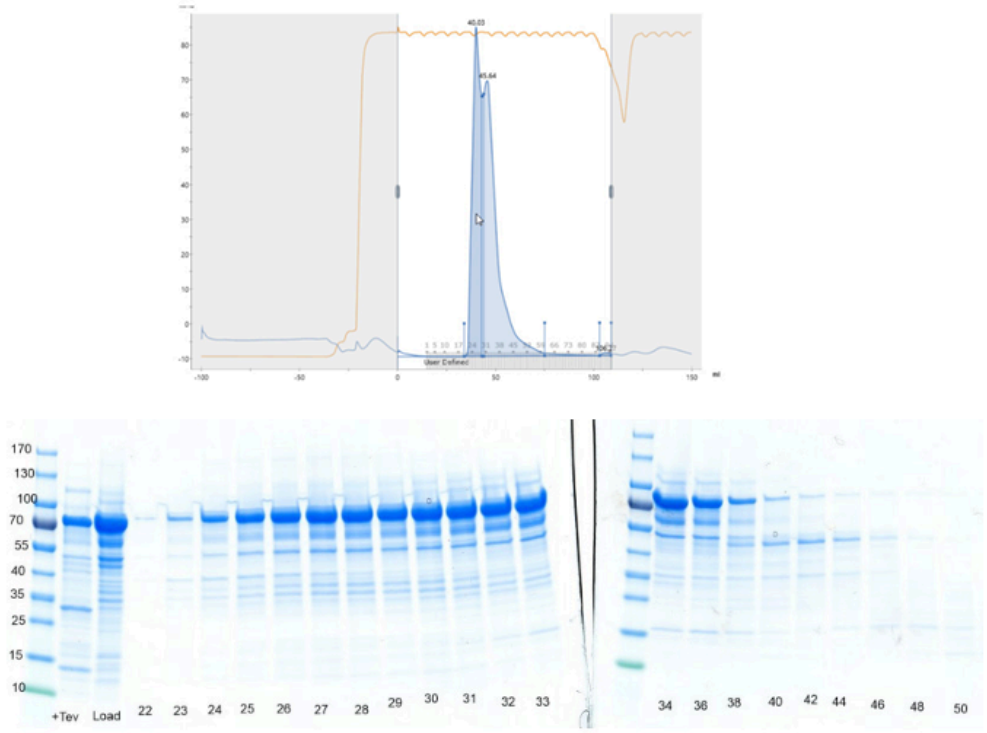
MonoQ chromatogram and SDS-PAGE analysis. Fractions of interest: 10 µL sample + 10 µL 2x SDS-PAGE sample buffer, loaded 10 µL. Red box: Pooled fractions (Fractions 33-44, 28 mL).

44 Concentrate the pooled fractions using a centrifugal ultrafiltration device (Sartorius VivaSpin 10 kDa) to 5 mL volume for size exclusion chromatography.

Size Exclusion Chromatography

45 Load the concentrated sample onto a Sephacryl S100 16/60 column (17-1165-01, Cytiva) previously equilibrated with SEC buffer.

46 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-PAGE sample buffer, loaded 15 µL. Collected eluted fractions (24-36, 13 mL).

47 Pool fractions containing Hsc70, concentrate to around 10 µL, aliquot and flash-freeze in liquid nitrogen for storage at -70 °C.