Jan 27, 2026

Zr-ELP purification protocol [Gravity columns]

DOI
https://dx.doi.org/10.17504/protocols.io.261ge1×2yv47/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.261ge1x2yv47/v1

Protocol Citation: Luis Lopez 2026. Zr-ELP purification protocol [Gravity columns]. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ge1x2yv47/v1

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

Created: January 27, 2026

Last Modified: January 27, 2026

Protocol Integer ID: 241654

Keywords: protein purification, elp purification protocol, using centrifugal column, centrifugal column, gravity column, denaturing buffer, zr

Abstract

Protein purification using centrifugal columns and denaturing buffers

Materials

- Lysis buffer (10 mM Tris, 6M Urea, pH 8)
- EDTA
- AEBSF (or PBSF)
- Ni-NTA resin
- Wash Buffer (10 mM Tris, 6M Urea, pH 6.3)
- Elution Buffer (10 mM Tris, 6M GuHCl, pH=8)
- Pierce spin column
- Falcon tubes
- Snakeskin dialysis tubing 6-8 kDa MWCO
- Dialysis buffer 10 mM Tris HCl pH=7.4
- DI water
- Amicon tube (MWCO 10 kDa)
- 2X SDS Loading buffer

Day 1 (1.5 hours)

1 FastTemp the big centrifuge to 4 C.

2 Resuspend the pellet cells in 40 ml of Lysis buffer (10 mM Tris, 6M Urea, pH 8). You can find it in the cold room.

3 Also add 1 mM EDTA and 1 mM AEBSF (or PBSF). These reagents are in the kitchen fridge in NSB. To calculate the volume to add, you can use this dilution calculator: https://www.sigmaaldrich.com/US/en/support/calculators-and-apps/solution-dilution-calculator?sitId=AfmBOooBbD4OXoY5sVzIFcaROHcIJWTMxEggx2luwbCA_xHIjyQNBUrotor.

4 Lyse in a sonicator. (1) Freezing-Thawing-Ultra sonification (with pulse 26 time mode: On 10s, Off 20s, Amplitude 50%, Time 10min) 2 cycles

5 Separate cells and proteins using a centrifuge (at 15,000 g for 30 min, 4 C) – Use the black, small rotor.

6 Keep the supernatant.

Purification (2 hours)

7 Pipette 2 ml of Ni-NTA resin into a Falcon tube and centrifuge it at 4000 rpm for 1 min.

8 Carefully remove ethanol excess. Resuspend the beads in 4 ml of lysis buffer, then centrifuge at 4000 rpm for 1 min.

9 Carefully remove supernatant. Resuspend beads in 2 ml of lysis buffer.

10 Incubate the clarified lysate supernatant from step 6 with Ni beads for 1 hour at 4 C in a rotator. Use a 50 ml Falcon Tube

11 Centrifuge beads (4000 rpm, 2 min, 4 C)

12 Carefully remove supernatant. Resuspend beads in 3 ml Wash Buffer (10 mM Tris, 6M Urea, pH 6.3).

13 Transfer the bead solution to a 10 ml Pierce spin column and place the columns into 50 ml Falcon tubes. The Pierce columns are on the shelf above the shared bench in NSB. Twist off the stopper. Do not throw it away.

14 Spin down at 200 rcf 4 C for 1 min. Collect 50 µL of the flow-through and discard the rest. Attach the plastic stopper.

15 Resuspend the beads in 5 ml of Wash Buffer in the Pierce column and centrifuge (200 rcf, 4 °C, 5 min). Keep 100 µL of the flowthrough. Discard the rest and attach the plastic stopper.

16 Repeat step 15 until Zr-ELP concentration is 3c 0.1 mg/ml [Use nanodrop MW: 7.76 kDa Ext. coeff./1000: 4.47]

17 Resuspend beads using 1 mL of Elution Buffer (10 mM Tris, 6M GuHCl, pH=8). Incubate for 10 min. Transfer the Pierce column to a clean 50 ml Falcon tube.

18 Elute Zr-ELP by spinning down 4C 200 rfc for 2 min. Retain flowthrough in a 15 ml tube.

19 Repeat steps 17 and 18 five times. [Total elution volume: 5 ml]

20 Measure protein concentration in the nanodrop for each elution fraction.

21 Add eluted protein to snakeskin dialysis 6-8 kDa MWCO.

22 Add the dialysis tubing in dialysis buffer 10 mM Tris HCl pH=7.4. Use a 1L beaker.

23 Change the dialysis buffer every four hours 4 times.

24 Place the dialysis tubing in DI water for 4 hours.

Day 3

25 Open the dialysis cassette and transfer the protein solution to a 10 mL Amicon tube (MWCO 10 kDa).

26 Centrifuge at 4000 rpm for 1 hour to concentrate the protein (4 C).

27 Measure protein concentration in the nanodrop.

28 Aliquot protein in 50 µL aliquot (PCR tubes)

29 Flash freeze in liquid nitrogen or dry ice. L

30 Transfer protein aliquot tubes to a labeled 50 mL tube (1 or 2).

31 Put at -80 °C.

Day 4

32 Prepare SDS Page samples.

33 Mix 15 uL of the sample with 15 uL of 2X SDS Loading buffer

34 Samples are:
- Unpurified cell lysate
- Flow through
- Wash
- 0.5 uL eluted
- 1 uL eluted
- 5 uL eluted