TALONspin™ Columns Protocol

PT5171-2

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I. Introduction

TALONspin™ Columns (Cat. Nos. 635601, 635602 & 635603) are ideal for rapidly and simultaneously purifying small amounts of his-tagged proteins under native or denaturing conditions. These columns, which are recommended for single-use applications, each contain 0.5 ml of TALON-NX Resin, which is optimized for performance in a spin column. When a clarified his-tagged protein extract obtained from fresh or frozen bacterial cell pellets is applied to a TALONspin column, his-tagged protein can be purified in approximately 30 minutes. Each column will yield 2–4 mg of his-tagged protein; exact yields will vary with conditions used and his-tagged protein characteristics. In addition, yield and purity will depend upon expression level and lysate concentration.

II. Buffer Preparation

Use the buffers supplied in the **HisTALON Buffer Set** (Cat. No. 635651) to extract and purify proteins under native and denaturing conditions (Section III) as follows:

A. Buffers for Extracting & Purifying Proteins under Native Conditions

- **Lysis Buffer:** use HisTALON xTractor Buffer
- **Equilibration Buffer:** use HisTALON Equilibration Buffer
- **Wash Buffer:** mix 660 µl HisTALON Elution Buffer with 9.34 ml HisTALON Equilibration Buffer
- **Elution Buffer:** use HisTALON Elution Buffer

B. Buffers for Extracting & Purifying Proteins under Denaturing Conditions

- **Equilibration Buffer:** add guanidine-HCl to HisTALON Equilibration Buffer to a final concentration of 6 M, and, if necessary, readjust the pH to 7.4.
- **Wash Buffer:** add guanidine-HCl to Wash Buffer (II.A) to a final concentration of 6 M, and, if necessary, readjust the pH to 7.4.
- **Elution Buffer:** add guanidine-HCl to HisTALON Elution Buffer to a final concentration of 5.4 M, and, if necessary, adjust the pH to 7.4.

III. Protein Extraction Protocols

IMPORTANT: We strongly recommend using **ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail** (Cat. No. 635673) when preparing your protein extract. Add 10 µl of Protease Inhibitor Cocktail per ml of lysis buffer [xTractor Buffer for native proteins (Section III.A) or denaturing Equilibration Buffer for denatured proteins (Section III.B)] **before** lysing cells to yield a 1X final concentration of inhibitors. For more information, refer to the ProteoGuard Protocol (type PT5140-2 in the keyword field at www.clontech.com/manuals).

A. Protocol: Sample Preparation to Isolate Native Proteins

This procedure has been optimized for extraction of native proteins from fresh or frozen bacterial cell pellet. The volumes of this extraction can be adjusted, as long as 20 ml of xTractor Buffer are used per 1 g of cell pellet.

1. Add 20 ml of xTractor Buffer to 1 g of bacterial cell pellet. Gently pipet up and down to fully resuspend the pellet.

   NOTE: A log-phase culture of *E. coli* (O.D.=0.6–0.8) when induced for 2–4 hours, would be expected to provide ~20–40 mg bacterial pellet from 2 ml of the culture.

2. **[Optional]**: Add 40 µl of 1 unit/µl DNase I solution and 200 µl of 100X lysozyme solution.

   NOTES:

   - DNase I reduces lysate viscosity, allowing more efficient cellular debris removal. DNase can be used without lysozyme—but cells with treated with lysozyme must also be treated with DNase I.
   - Lysozyme helps to fully disrupt bacterial walls, and thus to extract high molecular weight proteins (>40 KDa). However, lysozyme should be omitted for mammalian extraction procedures as well as when lysozyme interferes with your protein's functionality.
   - If the lysozyme solution forms a precipitate, resuspend the contents of the bottle and apply 200 µl of suspension directly to the mix—or (optionally) centrifuge 200 µl of lysozyme solution for 5 min at 14,000 rpm, and use the supernatant for the lysis.

3. Mix gently, pipetting up and down several times.
4. Incubate with gentle shaking for 10 min at room temperature or at 4°C.

   NOTE: At the end of this incubation period, there should be no visible particles. If cell pellet fragments are present, resuspend them by pipetting solution up and down and incubating for an additional 1–2 min.

5. Centrifuge the resulting cell lysate at 10,000–12,000 x g for 20 min at 4°C and carefully transfer the supernatant to a clean tube to yield the clarified sample.
6. Set aside a small portion of the clarified sample for SDS/PAGE analysis before starting TALONspin purification.

B. Protocol: Sample Preparation to Isolate Denatured Proteins

1. Harvest 20–25 ml of cell culture by centrifugation at 1,000–3,000 x g for 15 min at 4°C.
2. Resuspend the pellet in 2 ml of **denaturing** Equilibration Buffer (Section II.B) per 20–25 ml of culture.
3. Gently agitate or stir the sample until it becomes translucent.
4. Centrifuge the sample at 10,000–12,000 x g for 20 min at 4°C and carefully transfer the supernatant to a clean tube to yield the clarified sample.

5. Set aside a small portion of the clarified sample for SDS/PAGE analysis before starting TALONspin purification.

NOTE: Samples containing 6 M guanidinium must be dialyzed overnight against buffer containing 8 M urea before loading on a gel.

IV. Protein Purification Protocol

A. General Considerations for TALONSpin Column Purification

IMPORTANT: Follow these guidelines to achieve optimal results:

- Before proceeding with purification, estimate the concentration of his-tagged protein in your sample by running a sample of the clarified lysate directly on SDS/PAGE, and observing band intensity.
- Avoid excessively concentrated or viscous lysates.
- If the his-tagged protein concentration in the lysate is very dilute, use one column to enrich the protein from several 0.6–1 ml lysate aliquots as follows: Repeat Steps 9–15 (Section IV.B) until the desired amount of lysate has been processed—or concentrate your protein by reducing the sample volume.
- The centrifugation rotor and speed may affect your results. Ideally, you should centrifuge TALONspin Columns in a swinging bucket rotor to allow the sample to pass through the resin uniformly. However, a fixed angle rotor or a microcentrifuge is also acceptable. Centrifugation speeds higher than 700 x g may cause irregularities in the flow of solution through the resin bed, and thus, decrease the performance of the column.

B. Protocol: TALONSpin Column Purification

1. Hold the TALONspin Column upright and flick it until all resin falls to the bottom of the column. Then, snap off the breakaway seal.

NOTE: Save end-cap for later use.

2. Place column in the 2 ml collection tube.
3. Remove the clear top-cap and centrifuge column at 700 x g for 2 min to remove the storage buffer from the resin bed.

NOTE: The resin bed will appear semi-dry after centrifugation.

4. Remove column from centrifuge, and place the end-cap on the bottom of the column.
5. Add 1 ml of HisTALON Equilibration/ Buffer, close the column with the clear top-cap, and mix briefly to pre-equilibrate the resin.
6. Remove both caps from column and place column inside the 2 ml collection tube.
7. Recentrifuge at 700 x g for 2 min to pellet the resin. Discard the supernatant.
8. Repeat Steps 5–7, twice.
9. Place the end-cap on the bottom of the column and add 0.6–1 ml of the clarified sample from Section III.A or B to the resin. Close the column with the clear top-cap.
10. Allow sample to passively wet the resin bed for 30 sec.

11. Mix or vortex contents briskly for 1–2 sec, completely resuspending the resin in the lysate.
12. Gently agitate the suspension for 5 min to allow his-tagged protein to bind. Do not vortex.
13. Remove both caps from column and place column inside the 2 ml collection tube.
14. Centrifuge at 700 x g for 2 min.
15. Remove the column and collection tube from the centrifuge rotor, making sure that the entire sample has passed through the resin bed.

    NOTE: Viscous samples may require additional centrifugation.
16. Remove the flowthrough and save the 2 ml collection tube.
17. Place the 2 ml collection tube in the rotor.
18. Place the end-cap on the column, and add 1 ml of HisTALON Equilibration Buffer. Close the column with the clear top-cap.
19. Allow the buffer to passively wet the resin bed for 30 sec.
20. Agitate or vortex briskly for a few sec until the resin is completely resuspended.
21. Gently agitate for 5 min.
22. Remove both caps and place the column inside the 2 ml collection tube; then centrifuge at 700 x g for 2 min.
23. Repeat Steps 18–22. Repeat twice for particularly concentrated lysates, or if necessary, to improve purity.
24. Examine the resin bed to ensure that it appears semi-dry, and to ensure that all buffer has drained from the resin bed and the column end.
25. Discard the used 2 ml collection tube.
26. If necessary, repeat the spin to remove all traces of buffer.
27. Replace the end-cap on the spin column.
28. Add 400–600 µl of Elution Buffer. Close the column with the clear top-cap.

    NOTE: Alternatively, use 100 mM EDTA (pH 8.0) if it does not interfere with downstream applications of the protein. Samples eluted with EDTA will also contain cobalt.
29. Allow 1 min for Elution Buffer to passively wet the resin bed.
30. Briefly agitate or vortex to resuspend the resin.
31. Place a fresh 2 ml collection tube into the centrifuge rotor.
32. Remove both caps and place column into the 2 ml collection tube.
33. Centrifuge sample at 700 x g for 2 min.
34. Repeat Steps 27–33.

    NOTE: The his-tagged protein sample can generally be recovered in 800–1,200 µl of Elution Buffer, but it may be necessary to use a larger Elution Buffer volume or repeat Steps 27–33.
35. Determine his-tagged protein yield using gel or spectrophotometric analysis.

    NOTE: If the purity of the his-tagged protein preparation is unsatisfactory, refer to the procedure in the Troubleshooting Guide (Section V).

V. Troubleshooting Guide

Description of Problem	Possible Explanation	Solution
Excessive background after TALONspin Column protocol	Sample is too viscous	Dilute clarified sample with an equal volume of Equilibration/Wash Buffer and load as two aliquots.
	Loosely bound impurities are not being washed away prior to elution	1. Increase the number of 1 ml washes. 2. Use Wash Buffer to perform an intermediate wash step before elution. 3. To repurify a TALONspin sample, perform the following after determining his-tagged protein yield using gel or spectrophotometric analysis. a. Add 4 volumes of Wash Buffer to the semipurified sample. b. Load the sample onto another TALONspin Column. c. Wash twice with 1 ml of Wash Buffer. d. Elute as before (Section IV.B.27–33).
His-tagged proteins do not elute	Tag is not accessible under native conditions	Purify his-tagged protein under denaturing conditions.
	Elution Buffer is not optimal	• Elute with 150 mM imidazole or pH 4.0 buffer. • For proteins that will not elute otherwise, you can strip off the protein using 100 mM EDTA (pH 8.0). (This will remove the Co^{2+} from the resin and deposit it in your protein sample.) • Add 1–5 mM β-ME to reduce disulfide linkages. Supplement buffer with 1% nonionic detergent.

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