TALON™ Resins

I. List of Components

TALON, TALON Superflow, and TALON CellThru are supplied as 50% (w/v) slurries in nonbuffered 20% ethanol. Please note that during shipping and storage, the resin will settle; thus, we recommend that you thoroughly resuspend it before aliquotting. 2 ml of homogeneously resuspended resin will provide 1 ml of TALON Resin with a binding capacity of at least 5 mg of His-tagged protein.

Store TALON Resins, TALONspin Columns and TALON Buffers at 4°C. **Do not freeze.**

- **TALON™ Metal Affinity Resin**

Cat. #	Size
8901-1	10 ml
8901-2	25 ml
8901-3	100 ml
8901-4	250 ml

- **TALON™ Superflow Resin**

Cat. #	Size
8908-1	25 ml
8908-1	100 ml

- **TALONspin™ Columns** (#8902-1, -2, -3, -4)
  These columns contain 0.5 ml of TALON-NX™ Resin as a 50% suspension in nonbuffered 20% ethanol.

- **TALON™ CellThru**

Cat. #	Size
8910-1	10 ml
8910-2	100 ml

- **TALON™ CellThru Disposable Columns**
  2-ml column (#8914-1)
  10-ml column (#8915-1)

- **TALON™ Purification Kit** (#K1253-1)

10 ml	TALON Metal Affinity Resin
160 ml	5X Extraction/Wash Buffer (250 mM Sodium Phosphate, 1.5 M Sodium Chloride, pH 7)
160 ml	5X Extraction Buffer (250 mM Sodium Phosphate, 1.5 M Sodium Chloride, pH 8)
25 ml	10X Elution Buffer (1.5 M Imidazole, pH 7)
5	2-ml Disposable Gravity Columns
1	10-ml Disposable Gravity Column

- **TALON™ Buffer Kit** (#K1252-1)

160 ml	5X Extraction/Wash Buffer (250 mM Sodium Phosphate, 1.5 M Sodium Chloride, pH 7)
160 ml	5X Extraction Buffer (250 mM Sodium Phosphate, 1.5 M Sodium Chloride, pH 8)
25 ml	10X Elution Buffer (1.5 M Imidazole, pH 7)

- **TALON™ 2-ml Disposable Gravity Columns** (#8903-1)

II. Buffers for TALON™ Purification

A. Choosing Buffers

If you have not purchased the TALON Purification Kit (#K1253-1) or the TALON Buffer Kit (#K1252-1), we recommend preparing the following buffers for purifying His-tagged proteins under native or denaturing conditions. See Section III for preparing buffers with TALON Purification Kit or Buffer Kit. Before preparing other buffer compositions, please consult Reagent Compatibility Table to evaluate resin compatibility.

To decrease the amount of nonspecifically bound protein, we recommend using the Extraction/Wash Buffer at pH 7.0 during purification; however, if your target protein is more stable at pH 8.0, or if it does not adsorb at pH 7.0, use the Extraction Buffer at pH 8.0 (in place of the Extraction/Wash Buffer) during all extraction and wash steps. Note that at elevated pH values, amino acids other than histidine, as well as the peptide bond, contribute to protein adsorption. Thus, proteins without a polyhistidine tag can also adsorb to TALON Resins, which decreases resin specific capacity and the final purity of your target protein. You may choose to use either native or denaturing buffer conditions, depending on the solubility of your protein.

B. Native Buffers

Native protein purification regimens use buffer conditions that preserve the native, three-dimensional structure and surface charge characteristics of a selected soluble protein during harvest from an expression host. TALON's low affinity for nonpolyhistidine-tagged proteins minimizes contaminant carryover. In addition, increasing buffer ionic strength can minimize nonspecific interactions. Regardless of the conditions used and the nature of the polyhistidine-tagged protein being purified, most applications will benefit from the presence of 100–500 mM NaCl in the IMAC buffer. In many cases, adding glycerol or ethylene glycol neutralizes nonspecific hydrophobic interactions. Small amounts of nonionic detergent may also dissociate weakly bound species.

- **1X Extraction/Wash Buffer (pH** 7.0)

50 mM	Sodium Phosphate
300 mM	NaCl

- **1X Extraction Buffer** (pH 8.0)

50 mM	Sodium Phosphate
300 mM	NaCl

- **1X Elution Buffer**

Imidazole Elution (pH 7.0)

50 mM	Sodium Phosphate
300 mM	NaCl
150 mM	Imidazole

pH Elution (pH 5.0)

50 mM	Sodium Acetate
300 mM	NaCl

C. Denaturing Buffers

Denaturants, such as 6 M guanidinium, enhance protein solubility. Because overexpressed proteins in prokaryotic systems are sometimes insoluble, you may need to purify proteins under denaturing conditions. When purifying proteins under denaturing conditions, we recommend preparing buffers as indicated below.

In the presence of 6 M guanidinium, TALON's color will change from a pinkish-mauve to violet due to a change in metal ion hydration in response to a change in the chaotrope. After removal of the guanidinium, TALON will return to a pinkish-mauve color. The change to violet does not reflect any change in the physical or chemical binding properties of the resin. In fact, the color change can be useful for indicating the buffer in which the resin is suspended, and for following the movement of guanidinium through the resin bed.

- **1X Extraction/Wash Buffer** (pH 7.0)

50 mM	Sodium Phosphate
6 M	Guanidine-HCl
300 mM	NaCl

- **1X Extraction Buffer** (pH 8.0)

50 mM	Sodium Phosphate
6 M	Guanidine-HCl*
300 mM	NaCl

- **1X Imidazole Elution Buffer** (pH 7.0)

45 mM	Sodium Phosphate
5.4 M	Guanidine-HCl*
270 mM	NaCl
150 mM	Imidazole

D. Additional Buffers & Reagents

- **MES Buffer**

20 mM	2-(N-morpholine)-ethanesulfonic acid (MES), pH 5.0

- **5X SDS PAGE Sample Buffer**

15%	β-Mercaptoethanol (β-ME)
15%	SDS
50%	Glycerol
1.5%	Bromophenol blue

- **Phosphate Buffered Saline (PBS; pH 7.5)**

	Final conc.	To prepare 2 L of solution
Na_2HPO_4	58 mM	16.5 g
NaH_2PO_4	17 mM	4.1 g
NaCl	68 mM	8.0 g

Dissolve the above components in 1.8 L of deionized H_2O. Adjust to pH 7.5 with 0.1 N NaOH. Add deionized H_2O to final volume of 2 L. Store at room temperature

*Before SDS-PAGE analysis, guanidinium must be exchanged with 8 M urea.

III. TALON™ Kits Premade Buffers

If you have purchased the TALON Purification (#K1253-1) **or Buffer Kits** (#K1252-1), prepare buffers as described below. To decrease the amount of nonspecifically bound protein, we recommend using the Extraction/Wash Buffer at pH 7.0 during purification; however, if your target protein is more stable at pH 8.0, or if it does not adsorb to the resin at pH 7.0, use the Extraction Buffer (pH 8.0) in place of the Extraction/Wash Buffer during all extraction and wash steps. See page 38 and 42 for more information on choosing a buffer.

A. Extraction Buffer

1. Dilute one part of the 5X Extraction/Wash Buffer or 5X Extraction Buffer with four parts of deionized water.

2. Check and correct pH if necessary. The 1X Extraction/Wash Buffer should be at pH 7.0, while the 1X Extraction Buffer should be at pH 8.0.

B. Elution Buffer

Dilute one part of the 10X Elution Buffer with nine parts of 1X Extraction/Wash Buffer (pH 7.0) (or 1X Extraction Buffer [pH 8.0], depending on the solubility of your protein).

C. Denaturing Buffer

Add 6-M guanidinium to the Extraction/Wash Buffer (pH 7.0), or Extraction Buffer (pH 8.0), and the Elution Buffer prepared in Steps A and B, respectively.

Note: Perform all steps during the purification procedure in the presence of 6 M guanidinium. Unfortunately, protein samples containing high guanidinium concentrations form a precipitate when loaded on SDS -PAGE gels. Therefore, dialyze the sample overnight in a buffered solution containing 8 M urea before loading it onto the gel.

D. Wash Buffer

- In general, use the Extraction/Wash Buffer at pH 7.0 to wash nonadsorbed proteins. If the protein is not stable at pH 7.0, then use the Extraction Buffer at pH 8.0 with 5–10 mM imidazole.

- If your host cell line produces unwanted multi-histidine proteins, incorporate a more stringent wash:

  Dilute 5X Elution Buffer in either 1X Extraction/Wash Buffer or 1X Extraction Buffer for a final concentration of 5–10 mM imidazole (1:300–1:150 dilution).

  Note: If a small amount of precipitate is observed in the buffers, warm them at 37°C, and stir or shake to dissolve precipitate prior to diluting and using the buffers.

IV. General Considerations

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

A. General Information

1. Perform all manipulations at 4–8°C in order to maintain protein stability and improve yield.

2. This protocol is designed using the Extraction/Wash Buffer (pH 7.0). If your target protein is more stable at pH 8.0, or if it does not adsorb at pH 7.0, use the Extraction Buffer at pH 8.0 (instead of the Extraction/Wash Buffer) during extraction and wash steps.

3. A reducing agent, such as 10 mM β-ME, or a protease inhibitor, such as PMSF, in the Extraction/Wash Buffer (pH 7.0), may improve the structural stability of fragile proteins during sample preparation. See Reagent Compatibility Table for more compatibility information.

   Note: Depending on the concentration and volume of the additive you wish to use, you may need to remake the buffers to preserve the recommended concentration of NaCl and buffering agent. DTT and DTE are not compatible with this TALON protocol at any concentration.

4. If the cell lysate contains a high level of proteolytic activity, we recommend adding 1 mM EDTA to the Extraction/Wash Buffer (pH 7.0) to inhibit metallo proteases during the extraction. **However, before applying the sample to TALON resin, remove EDTA using a gel filtration column (PD-10, Amersham, Pharmacia) equilibrated with the Extraction/Wash Buffer.** In some cases, the host cell produces low molecular weight chelators that can also be removed using gel filtration.

   Chelators can be detected easily by applying your sample to a small column packed with TALON Resin. If the top of the column loses its characteristic pink color, and the colorless front moves in the direction of the flow, or if you obtain pink fractions during batch adsorption, you must equilibrate the sample using a gel filtration column.

5. Overexpressed recombinant proteins can accumulate in insoluble inclusion bodies. In order to determine optimal extraction/purification conditions, you must determine the distribution of the protein in soluble and insoluble forms. Perform a preliminary SDS-PAGE analysis of protein extracts obtained under native conditions, followed by extraction of the residual proteins under denaturing conditions. Take care to use the same extraction volumes for both native and denaturing extracts, and run the cell extract before induction as a control in one lane to identify the target protein. Use of denaturing conditions is recommended only if the biological activity of the target protein is not affected by denaturation. In that case, it is preferable to use native conditions for extraction even if only 5–10% of the target protein is soluble.

6. The buffer volumes in the following protocols were optimized for purifying the HAT-DHFR protein from 20–25 ml of *E. coli* culture.

   Depending on the expression level and anticipated yield, you may need to adjust the buffer volumes for other proteins. As a starting point, use 2 ml of buffer per 20–25 ml of culture.

7. If you are purifying protein from harvested eukaryotic cells, lyse the cells in an appropriate buffer containing a mild detergent (Sambrook *et al.*, 1989). See Reagent Compatibility Table for compatible buffer additives.
   Note that EDTA and EGTA are not compatible with the TALON Resins because these reagents strip the cobalt from the resin.

8. Carefully check the sample appearance after lysis or sonication. Bacterial samples often remain viscous from incomplete shearing of genomic DNA. Complete DNA fragmentation improves protein yields and allows efficient removal of cellular debris during centrifugation. You may decrease the sample viscosity by digestion for 20–30 min at room temperature with 2.5 µg/ml of DNase I. Remember that proteolytic activity is much higher at room temperature. Alternatively, dilute the sample five fold with Extraction/Wash Buffer before applying it to the resin. This procedure should not significantly affect recovery.

9. Use 2 ml of resin suspension per ~3 mg of anticipated polyhistidine-tagged protein. 2 ml of homogeneously resuspended resin will provide 1 ml (bed volume) of TALON Resin.

10. The buffers and purification conditions should work well for most soluble, monomeric proteins expressed in *E. coli*.

11. Initially, test each different expression system and polyhistidine-tagged protein in small-scale batch purification to determine expression levels and to optimize the protocol (See Section V).

B. Elution Strategy: Imidazole vs. pH Gradient Elution

TALON purification schemes typically use either an imidazole or a pH gradient for washing and elution. Imidazole in the Extraction and/or Extraction/Wash Buffers minimizes nonspecific binding and reduces the amount of contaminating proteins. Thus, we recommend first purifying polyhistidine-tagged proteins using an imidazole gradient. However, imidazole and polyhistidine-tagged proteins absorb at 280 nm and elution peaks may be difficult to detect spectrophotometrically, especially if you are purifying small amounts of polyhistidine-tagged proteins. In these cases, collect the leading edge of the imidazole breakthrough peak and check for polyhistidine-tagged proteins by a protein specific assay (Bradford, 1976) and SDS-PAGE. Alternatively, use a pH gradient to purify polyhistidine-tagged proteins that are stable from pH range 5.0–7.0. See Section II for buffer compositions.

C. Elution Strategy: Step vs. Linear Gradients

In most cases, step gradients are preferred over linear gradients, because linear gradients lead to broader elution peaks, which can dilute the product and make detection more difficult. In addition, scaling-up step gradients is less complicated than scaling-up linear gradients.

D. Reusing TALON™ Resins

TALON Resins may be stored and reused up to 3–4 times before discarding or complete regeneration; the exact number of uses depends on the application. To avoid possible cross-contamination, use a particular aliquot of resin for purifying a single type of His-tagged protein. See Section VIII for important information on washing, storing, and reusing TALON Resins.

E. TALON™ CellThru Considerations

The procedure for purifying His-tagged proteins using TALON CellThru is similar to methods for other TALON Resins with the following significant differences.

1. **Extracellular Proteins**
   If there are no chelating agents in the fermentation liquid and the pH is ≥7.0, you can apply the sample directly to a TALON CellThru-prepacked column. Otherwise, a desalting/equilibration step by ultracentrifugation or gel filtration with Sephadex G25 is necessary.

2. **Intracellular Proteins**
   For purifying intracellular proteins, apply the sonicated sample containing your target proteins directly to a TALON CellThru-prepacked column. There is no need for centrifugation. Electrophoresis may reveal that some of the target protein has passed through the column without adsorption. To a large extent, the unabsorbed protein is an insoluble material which is normally removed during high-speed centrifugation. The amount of non-adsorbed target protein will vary as a function of sonication efficiency.

3. **Chromatography Considerations**
   TALON CellThru Beads have a diameter of 300–500 µm; therefore, use a column with a filter pore size of 90–130 µm to adequately pass cellular debris. We recommend using our CellThru 2-ml & 10-ml Disposable Columns (#8914-1 & #8915-1). The 2-ml columns are suitable for 1–2-ml bed volumes, while the 10-ml columns are suitable for 5–10 ml bed volumes. Because the column filters have a larger pore size and permit higher flow rates, you may need to incubate your sample with the adsorbent for 5 minutes before letting it flow through. If necessary, pass the sample through the column a second time.

V. Test Expression Levels and Purification Strategy

A. Miniscale purification

Mini-scale protein purification is ideal for any of the following:

- checking for a His-tagged protein
- determining expression levels
- testing buffer conditions

You can also use a TALONspin Column (#8902-1) with this procedure (p. 45).

We recommend that you set aside a sample after each critical step of the procedure, and analyze all samples by SDS-PAGE.

Important Notes

- This protocol is not intended for obtaining highly purified His-tagged protein samples. Furthermore, protein samples eluted with EDTA (Step 19, below) will contain cobalt and EDTA, which may inhibit enzyme activity as well as cause the protein to precipitate.
- This protocol was optimized using denaturing conditions at pH 8.0. If you wish to obtain native samples, then substitute buffers accordingly. You may also need to use lysozyme (0.75 mg/ml of native buffer) to completely disrupt the cells in Step 5.

1. Transfer 1 ml of expression culture to a 1.5-ml microcentrifuge tube.
2. Centrifuge at 14,000 rpm for 2 min.
3. Remove and discard supernatant.
4. Add 0.5 ml of Denaturing Extraction Buffer (pH 8.0).
5. Vortex until cell pellet is completely dissolved.
6. Centrifuge at 14,000 rpm for 5 min to pellet any insoluble debris.
7. Set aside 50 µl of the supernatant for later analysis. Transfer the remainder of the supernatant that was prepared as described in Section VI.B.1–6 to a clean 1.5-ml tube containing 50 µl of prewashed TALON Resin, . Start with 100 µl of resuspended TALON Resin slurry.
8. Agitate sample at room temperature for 10 min.
9. Centrifuge at 14,000 rpm for 1 min to pellet protein/resin complexes.
10. Carefully remove the supernatant and set aside 50 µl for later analysis. A high protein concentration in this sample indicates a problem with protein binding.
11. Add 1 ml of Denaturing Extraction Buffer.

12. Vortex for a few seconds.

13. Centrifuge at 14,000 rpm for 1 min to pellet resin.

14. Remove the supernatant and set aside 50 µl ("first wash") for later analysis. Discard the remainder of the supernatant.

15. Repeat Steps 11–14. Set aside 50 µl for analysis.

16. Elute bound polyhistidine-tagged protein by adding 50 µl of Elution Buffer to the resin/protein pellet and briefly vortexing.

17. Centrifuge briefly at 14,000 rpm.

18. Carefully remove the supernatant containing the polyhistidine-tagged protein.

19. Repeat Steps 16–18. Alternatively, if you only intend to determine the concentration of polyhistidine-tagged protein in your sample, you can achieve a more complete elution and thus, a more accurate protein quantification by eluting with EDTA as follows:

    a. Add 50 µl of 100 mM EDTA (pH 8.0) and vortex briefly.

    b. Centrifuge briefly at 14,000 rpm.

    c. Carefully remove the supernatant containing the 6xHis protein

    Note: EDTA removes bound metal from the resin; the protein sample will contain cobalt, and the TALON Resin cannot be reused unless completely regenerated as described in Section VIII.D.

20. Add 12 µl of 5X SDS-PAGE Sample Buffer to each of the saved samples.

    Note: The sample buffer will reduce multimers to monomers; thus, only a single band will be visible on an SDS-PAGE gel, even for naturally homologous multimeric proteins.

21. Heat samples at 95–98°C for 5 min.

22. Load samples and analyze on an SDS-PAGE gel.

B. TALONspin™ Column Purification

Important

- Before proceeding with purification, determine the concentration of polyhistidine-tagged protein in your sample using the miniscale procedure (Section V. A). Alternatively, run a sample of the clarified lysate directly on SDS-PAGE, and estimate the amount of polyhistidine-tagged protein by band intensity.

- Avoid excessively concentrated or viscous lysates. See Troubleshooting Guide for tips on reducing sample viscosity.

- If the concentration of His-tagged protein in the lysate is very dilute, use one column to enrich the protein from several 0.6–1-ml lysate aliquots. Simply repeat Steps 7–13 (below) until the desired amount of lysate has been processed. Alternatively, concentrate the His-tagged protein by reducing the sample volume.
- The centrifugation rotor and speed may affect your results. Ideally, 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 micro-centrifuge 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.

1. Hold the TALONspin Column upright and flick it until all resin falls to the bottom of the column.
2. Snap off the breakaway seal.
3. Place column in the 2-ml microcentrifuge tube.
4. Save white end-cap for later use.
5. 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.
6. Remove column from centrifuge, and place the white end-cap over the male luer fitting.
7. Add 1-ml 1X Extraction/Wash Buffer and mix briefly to pre-equilibrate the resin.
8. Re-centrifuge at 700 x g for 2 min to pellet the resin. Discard the flowthrough.
9. Repeat Steps 7 and 8, twice.
10. Add the clarified sample from Section V.A or V.B to the resin.
11. Add 0.6–1 ml of sample to the column, and replace the clear top-cap.
12. Allow sample to passively wet the resin bed for 30 sec.
13. Mix or vortex contents briskly for a 1–2 sec, completely resuspending the resin in the lysate.
14. Gently agitate the suspension for 5 min to allow His-tagged protein binding. **Do not vortex.**

15. Remove both caps from column and place column inside the 2-ml microcentrifuge tube.
16. Centrifuge at 700 x g for 2 min.
17. Remove the column and microcentrifuge tube from the centrifuge rotor, making sure that all of the sample has passed through the resin bed.

    Note: Viscous samples may require additional centrifugation.

18. Discard the flowthrough, but save the 2-ml tube.
19. Place microcentrifuge tube in rotor.
20. Place white end-cap on the column, and add 1 ml of 1X Extraction/Wash Buffer. Close the column with the clear top-cap.
21. Allow the buffer to passively wet the resin bed for 30 sec.
22. Agitate or vortex briskly for a few seconds until the resin is completely resuspended.
23. Gently agitate for 5 min.
24. Remove both caps and centrifuge at 700 x g for 2 min.
25. Repeat Steps 18–24. Repeat twice for particularly concentrated lysates, or if necessary, to improve purity.
26. Examine the resin bed to ensure that it appears semidry, and to ensure that all wash buffer has drained from the resin bed and the column end.
27. Discard the used 2-ml microcentrifuge tube.
28. If necessary, repeat the spin to remove all traces of wash buffer.
29. Replace the white end-cap on the spin column.
30. Add 400–600 µl of Elution Buffer.

    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.

31. Allow 1 min for Elution Buffer to passively wet the resin bed.
32. Briefly agitate or vortex to resuspend the resin.
33. Place a fresh 2-ml collection tube into centrifuge rotor.
34. Remove both caps and place column into the 2-ml collection tube.

35. Centrifuge sample at 700 x g for 2 min.

36. Repeat Steps 30–35.

    Note: The polyhisidine-tagged protein can usually be recovered in 800–1200 µl of Elution Buffer. If necessary, use a larger Elution Buffer volume or repeat Steps 30–35.

37. Determine polyhistidine-tagged protein yield using gel or spectrophotometric analysis.

VI. Sample Preparation

A. Native Proteins

This procedure can be used with any TALON Resin and TALON Superflow Resin. For CellThru Sample preparation, see Section VI. C.

If this is the first time you have prepared clarified samples from cells expressing a particular recombinant protein, we recommend that you estimate the protein's expression level in that host strain. To do so, perform a mini-scale purification, and then analyze a portion by SDS-PAGE in parallel with protein standards. Once satisfactory expression is observed, proceed with the appropriate purification protocol.

1. Harvest the cell culture by centrifugation at 1,000–3,000 x g for 15 min at 4°C. Remove the supernatant. If yield is low, use the mild extraction method described in Step 6.

2. Resuspend the cell pellet by vortexing in 2 ml of chilled 1X Extraction/Wash Buffer (4°C) per 25 ml of culture ≤100 ml. For cultures >1 L, resuspend the pellet in 1–2% of the original culture volume.

   Note: You may omit Steps 3–4 if lysozyme treatment interferes with your protein's functionality.

3. Add lysozyme to the 1X Extraction/Wash Buffer for a concentration of 0.75 mg/ml. To reduce the chance of introducing proteases, use the highest purity lysozyme available.

4. Incubate at room temperature for 20–30 min.

   Note: Incubations at room temperature result in elevated proteolytic activities. Alternatively, you can use lysozyme at 4°C with lower efficiency. If this treatment hydrolyzes the target protein, use the method described in Step 6. Alternatively, disrupt the cells by repeated freeze/thaw cycles; that is, flash-freezing the cell suspension in a dry ice-ethanol bath and thawing in chilled H_2O.

5. If your sample is ≤50 ml, sonicate it 3 x 10 sec, with a pause for 30 sec on ice between each burst. If your sample is ≥ 200 ml, sonicate it 3 x 30 sec, with a 2 min pause on ice between each burst. Proceed to Step 7.

   Note: Excessive sonication can destroy protein functionality.

6. [Optional]: **High-yield, mild extraction method.** Transfer the cells to a chilled mortar and grind 1 part cells with 2.5 parts Alumina (Sigma, #A-2039) for 2–3 min or until the composition of the mixture becomes paste-like. Add 2 ml chilled 1X Extraction/Wash Buffer (4°C) per 25 ml culture.

   Note: If there is a high level of proteolytic activity in the cell lysate, we recommend adding 1 mM EDTA (final concentration) to the Extraction/Wash Buffer in order to inhibit metallo-proteases during the extraction. Before application of the sample to the TALON adsorbent, EDTA must be removed by gel filtration chromatography (PD-10, Amersham, Pharmacia) equilibrated with the Extraction/Wash Buffer for IMAC.

7. Centrifuge the cell extract at 10,000–12,000 x g for 20 min at 4°C to pellet any insoluble material.

8. Carefully transfer the supernatant to a clean tube without disturbing the pellet. This is the clarified sample.

9. Store a small portion of the clarified sample at 4°C for SDS-PAGE analysis.

B. 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 1X Extraction/Wash Buffer (pH 7.0) 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 to pellet any insoluble material.

5. Carefully transfer the supernatant to a clean tube without disturbing the pellet. This is the clarified sample.

6. Set aside a small portion of the clarified sample for SDS-PAGE analysis. Then proceed with the appropriate purification protocol (below).

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

C. TALON™ CellThru Sample Preparation

Native Proteins

1. Harvest the cell culture by centrifugation at 1,000–3,000 x g for 15 min at 4°C. Remove the supernatant. If yield is low, use the mild extraction method described in Step 6.

2. Resuspend the cell pellet by vortexing in 2 ml of chilled 1X Extraction/Wash Buffer (4°C) per 25 ml of culture ≤100 ml. For cultures >1 L, resuspend the pellet in 1–2% of the original culture volume.

   Note: You may omit Steps 3–4 if lysozyme treatment interferes with your protein's function.

3. Add lysozyme to the 1X Extraction/Wash Buffer for a concentration of 0.75 mg/ml. To reduce the chance of introducing proteases, use the highest purity lysozyme available.

4. Incubate at room temperature for 20–30 min.

   Note: Incubations at room temperature result in elevated proteolytic activities. Alternatively, you can use lysozyme at 4°C with lower efficiency. If this treatment hydrolyzes the target protein, use the method described in Step 6. Alternatively, disrupt the cells by repeated freeze/thaw cycles; that is, flash-freezing the cell suspension in a dry ice-ethanol bath and thawing in chilled H_2O.

5. If your sample is ≤50 ml, sonicate it 3 x 10 sec, with a pause for 30 sec on ice between each burst. If your sample is ≥ 200 ml, sonicate it 3 x 30 sec, with a 2-min pause on ice between each burst.

   Note: Excessive sonication can destroy protein functionality.

6. Store a small portion of the clarified sample at 4°C for SDS-PAGE analysis.

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 1X Extraction/Wash Buffer (pH 7.0) per 20–25 ml of culture.

3. Gently agitate or stir the sample until it becomes translucent

4. Set aside a small portion of the clarified sample for SDS-PAGE analysis. Then proceed with the appropriate purification protocol (Section VII).

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

VII. Protein Purification Protocols

A. Batch/Gravity-Flow Column Purification

For IMAC column using TALON, we recommend a hybrid batch/gravity-flow procedure. This method combines the speed and convenience of a batch procedure with the higher purity of the gravity-flow column method. In this hybrid procedure, the binding and initial washing steps are performed in a batch format to save time, eliminate extraneous debris, and avoid column clogging. After the initial washes, the resin is transferred to a column for additional washing and protein elution.

1. Thoroughly resuspend the TALON Resin.
2. Immediately transfer the required amount of resin suspension to a sterile tube that will accommodate 10–20 times the resin bed volume.
3. Centrifuge at 700 x g for 2 min to pellet the resin.
4. Remove and discard the supernatant.
5. Add 10 bed volumes of 1X Extraction/Wash Buffer and mix briefly to pre-equilibrate the resin.
6. Re-centrifuge at 700 x g for 2 min to pellet the resin. Discard the supernatant.
7. Repeat Steps 5 and 6.
8. Add the clarified sample from Section VI to the resin.
9. Gently agitate at room temperature or on ice*for 20 min on a platform shaker to allow the His-tagged protein to bind the resin.
10. Centrifuge at 700 x g for 5 min.
11. Carefully remove as much supernatant as possible without disturbing the resin pellet.
12. Wash the resin by adding 10–20 bed volumes of 1X Extraction/Wash Buffer. Gently agitate the suspension at room temperature or on ice for 10 min on a platform shaker to promote thorough washing.
13. Centrifuge at 700 x g for 5 min.
14. Remove and discard the supernatant.
15. Repeat Steps 12–14.

16. Add one bed volume of the 1X Extraction/Wash Buffer to the resin, and resuspend by vortexing.

    Note: Steps 17–22 can be performed on ice or at room temperature*.

17. Transfer the resin to a 2-ml gravity-flow column with an end-cap in place, and allow the resin to settle out of suspension.

18. Remove the end-cap and allow the buffer to drain until it reaches the top of the resin bed, making sure no air bubbles are trapped in the resin bed.

19. Wash column once with 5 bed volumes of 1X Extraction/Wash Buffer.

20. **[Optional]:** If needed, repeat Step 19 with more stringent conditions using 5–10 mM imidazole in 1X Extraction/Wash Buffer (Section II.C).

21. Elute the polyhistidine-tagged protein by adding 5 bed volumes of Elution Buffer to the column. Collect the eluate in 500-µl fractions.

    Note: Under most conditions, the majority of the His-tagged protein will be recovered in the first two bed volumes.

22. Use spectrophotometric and SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

    Note: Use a Bradford protein assay (Bradford, 1976) or UV absorbance at 280 nm. Use UV absorbance only if you are eluting sufficient protein to exceed the absorbance of the imidazole at 280 nm. Alternatively, you might dialyze the fractions overnight against the Extraction/Wash Buffer, and then measure their UV absorbance at 280 nm.

* Incubation on ice will decrease proteolysis

B. Large-Scale Batch Purification

This method purifies polyhistidine-tagged proteins faster than gravity-flow columns; however, batch washes remove impurities less efficiently than gravity-flow columns. Therefore, they require larger wash buffer volumes to obtain pure polyhistidine-tagged proteins.

1. Thoroughly resuspend the TALON Resin.
2. Transfer the required amount of resin to a glass filter with a pore size of 10–20 µm.
3. Apply a vacuum to the filter to remove excess ethanol.
4. Add 5 bed volumes of deionized water to the resin and apply vacuum.
5. Add 5 bed volumes of 1X Extraction/Wash Buffer to the resin and apply vacuum.
6. Repeat Step 5 two times.
7. Add crude lysate (TALON CellThru) or clarified sample (TALON & TALON Superflow) to the resin and mix for 3–5 min.
8. Apply vacuum and collect the filtrate.
9. Wash the resin by adding 10–20 bed volumes of 1X Extraction/Wash Buffer. Gently agitate the suspension at room temperature for 10 min on a platform shaker to promote thorough washing.
10. Apply vacuum to remove buffer.
11. Repeat the above wash (Steps 9–10) 2–3 times.
12. [Optional]: If necessary, repeat Step 11 under more stringent conditions using 5 mM imidazole in 1X Extraction/Wash Buffer (Section II. C.).
13. Elute the polyhistidine-tagged protein by adding 5 bed volumes of Elution Buffer.
14. Gently agitate suspension at room temperature for 5 min.
15. Apply vacuum, and collect the purified polyhistidine-tagged protein.
16. Repeat Steps 13–15 two times, collecting separate fractions.
17. Use spectrophotometric and SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

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

C. Medium-Pressure Column (FPLC) Purification Using TALON™ Superflow

1. Assemble column according to the manufacturer's instructions.

2. Thoroughly resuspend TALON Superflow Resin. Slowly pour the slurry into the column, and avoid introducing air bubbles.

3. Allow resin to settle. Accelerate this process by allowing the buffer to flow through the column with a peristaltic pump attached to the output of the column. Do not exceed a flow rate of 5 ml/min/cm^2. Do not allow the resin to dry out. If this occurs, resuspend the resin and repack the column.

4. Add deionized water to the top of the column to avoid trapping air between the adapter and the resin surface.

5. Insert and adjust the top adaptor. Then, connect the column to the chromatography system.

6. Equilibrate the column with 1X Extraction/Wash Buffer. Do not exceed a 5 ml/min/cm^2 flow rate. Monitor the eluant at 280 nm; the baseline should be stable after washing with 5–10 column-volumes.

7. Apply the clarified sample to the column after filtering it through a 0.22-µm filter and wash with Extraction/Wash Buffer until the baseline (280 nm) is stable. Monitor column backpressure during sample application. Start collecting fractions.

   Note: If the sample is very viscous, the column pressure may start to exceed the recommended value (150 psi, 1.0 MPa). If this occurs, then reduce the flow rate or dilute the sample to bring the pressure into an acceptable range.

   Load the sample at a flow rate of 0.5–1.0 ml/min/cm^2 to ensure that the polyhistidine-tagged protein will bind to the resin. If the protein does not bind, reduce the flow rate further. If desired, increase the flow rate for washing and protein elution.

   If the target protein is unstable at room temperature, perform the chromatography at 4°C. Alternatively, use flow rates up to 5 ml/min/cm^2 to load, wash, and elute the protein. Capacity will decrease by 10–15%, but on average, a chromatography run should only take 15–20 min.

8. Wash the column with 10–20 column volumes of Extraction/Wash Buffer, or until the baseline at 280 nm is stable. If necessary, wash with 5–10 mM imidazole in Extraction/Wash Buffer.

9. Elute the polyhistidine-tagged protein with 5–10 column-volumes of Elution Buffer. The polyhistidine-tagged protein usually elutes in the second and third column-volumes.

10. Use spectrophotometric and SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

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

11. If you plan to store, regenerate, and reuse a TALON Superflow-packed Column, see Section VIII.C.

VIII. TALON Resin Regeneration and Storage

Generally, reuse TALON Resins 3–4 times before discarding. The exact number of uses varies among preparations because of differences in redox potential, organic complexity, and debris content. To avoid possible cross-contamination, use a particular aliquot of resin to purify a single type of polyhistidine-tagged protein.

Important precautions

- TALONspin Columns are not reusable.
- Do not store TALON Resin in denaturants such as 6 M guanidinium.
- Do not store TALON Resin with bound imidazole; the resin should be washed with 2-(N-morpholine)-ethanesulfonic acid (MES) buffer (pH 5.0) before reuse to remove the bound imidazole.

A. Stringent Wash (Optional)

1. Wash resin with four bed volumes of 6 M guanidinium (pH 5.0) and 1% nonionic detergent.
2. Rinse resin with five bed volumes of distilled H_2O.
3. Store resin at 4°C in 20% nonbuffered ethanol containing 0.1% sodium azide.

B. Removing Imidazole

1. Wash resin with five bed volumes of 20 mM MES buffer (pH 5.0) containing 0.1 M NaCl.
2. Rinse resin with five bed volumes of distilled H_2O.
3. Store resin at 4°C in 20% nonbuffered ethanol containing 0.1% sodium azide.

C. Regeneration of TALON™ Superflow Columns

Purification of His-tagged proteins using imidazole gradients will cause the column to take on a purplish hue. Washing the column with 5–10-column volumes of 20 mM MES buffer (pH 5.0) will restore the normal pink color and bring the absorbance at 280 nm back to the original baseline level. After equilibrating the column with Extraction/Wash Buffer, the column is ready for reuse.

D. Complete Regeneration

Strip the resin of cobalt ions by washing with 10 bed volumes of 0.2 M EDTA (pH 7.0). Wash excess EDTA with an additional 10 bed volumes of Milli-Q H_2O. Charge the resin with 50 mM $CoCl_2$ solution (10 bed volumes). Again, wash with 10 bed volumes of Milli-Q H_2O to remove excess cobalt metal ions. Equilibrate the resin with extraction/wash buffer (10 bed volumes).

E. Regeneration and re-use of TALON™ and TALON™ Superflow resins

The regeneration and re-use of TALON and TALON Superflow Resins depends on the nature and the presence of subcellular particles, hydrophobic proteins, and DNA/RNA in the samples that are applied to the resin. We recommend that this regeneration procedure is not repeated more than 5 times and is used for purification of only one type of protein.

1. Wash the column with five column volumes of 50 mM sodium phosphate; 0.25 M NaCl; 0.3 M imidazole pH 7.5.
2. Wash the column with three column volumes of deionized H_2O.
3. Wash the column with five column volumes of 25% ethanol†.
4. Wash the column with three column volumes of deionized water.
5. Wash the column with five column volumes of 0.2 M EDTA (pH 7.5).
6. Wash the column with 10 column volumes of deionized water.
7. Charge the resin with three column volumes of 100 mM $CoCl_2 \bullet 6H_2O$.
8. Wash the column with ten column volumes of deionized H_2O.
9. Equilibrate the column with 10 column volumes of the respective sonication/loading buffer. Check the pH of the flow through—it has to be the same as that of your buffer.

*All solutions for regeneration of FPLC columns have to filtered through a 0.22-µm filter and degassed before use. Only the 25% ethanol and, if used, the 30% n-propanol has to be degassed when used for regeneration of low pressure/gravity flow columns. The batch procedure can be performed using the same washing steps on a sintered glass filter.

† If the yield from the material balance from the previous chromatography run on the column is lower than 80%, we suggest that the column is washed additionally with 30% n-propanol after the wash with 25% ethanol. This will remove most of the hydrophobic proteins that were adsorbed nonspecifically to the resin. Repacking of the column might be necessary if air accumulates in the column bed during this step. FPLC columns packed with TALON Superflow can be degassed by washing them at elevated flow rates of up to 15 cm/min linear flow rate during Step 6 until all air bubbles are washed out. Do not exceed backpressure of 150 psi (1.0 MPa).

Linear flow rate (cm/min) is the volumetric flow rate (ml/min) divided by the cross section area of the column (πr^2) in cm^2, where $\pi = 3.1416$ and r is the radius of the cross section of the column in cm.