Protocol-at-a-Glance

# HisTALON™ Gravity Column Protocol-at-a-Glance

**(PT3956-2)**

This Protocol-at-a-Glance provides general instructions for the purification of recombinant his-tagged proteins using the HisTALON Gravity Column (Cat. No. 635645). The column can be used under native or denaturing conditions with the buffers described in Section B and the protocols outlined in Sections C–E. For more detailed information regarding protein purification, as well as protein expression (transformation of host cells with expression vectors), please consult the TALON® Metal Affinity Resins User Manual (PT1320-1) at **www.clontech.com**

## A. Column Capacity

Each HisTALON Gravity Column contains 1 ml (bead volume) of TALON Resin (Cat. No. 635501). Each column will bind 5 mg of his-tagged protein.

## B. Choosing Buffers

To decrease the amount of nonspecifically-bound proteins, we recommend using the Equilibration/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 Equilibration Buffer at pH 8.0 (in place of the Equilibration/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 his tag can also adsorb to IMAC resins, which decreases resin 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. Figure 1 outlines the purification procedure.

### 1. Native Buffers

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

  50 mM sodium phosphate
  300 mM NaCl

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

  50 mM sodium phosphate
  300 mM NaCl

- **1X Elution Buffer***

| – Imidazole Elution (pH 7.0) | | | – pH Elution (pH 5.0)[1] | | |
|---|---|---|---|---|---|
| 50 | mM | sodium phosphate | 50 | mM | sodium acetate |
| 300 | mM | NaCl | 300 | mM | NaCl |
| 150 | mM | imidazole | | | |

[1]Prepare fresh before use.

### 2. Denaturing Buffers

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

  50 mM sodium phosphate
  6 M guanidine-HCl
  300 mM NaCl

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

  50 mM sodium phosphate
  6 M guanidine-HCl
  300 mM NaCl

* See Sambrook, Appendix B.21, or your standard protocol for preparing sodium phosphate buffer.

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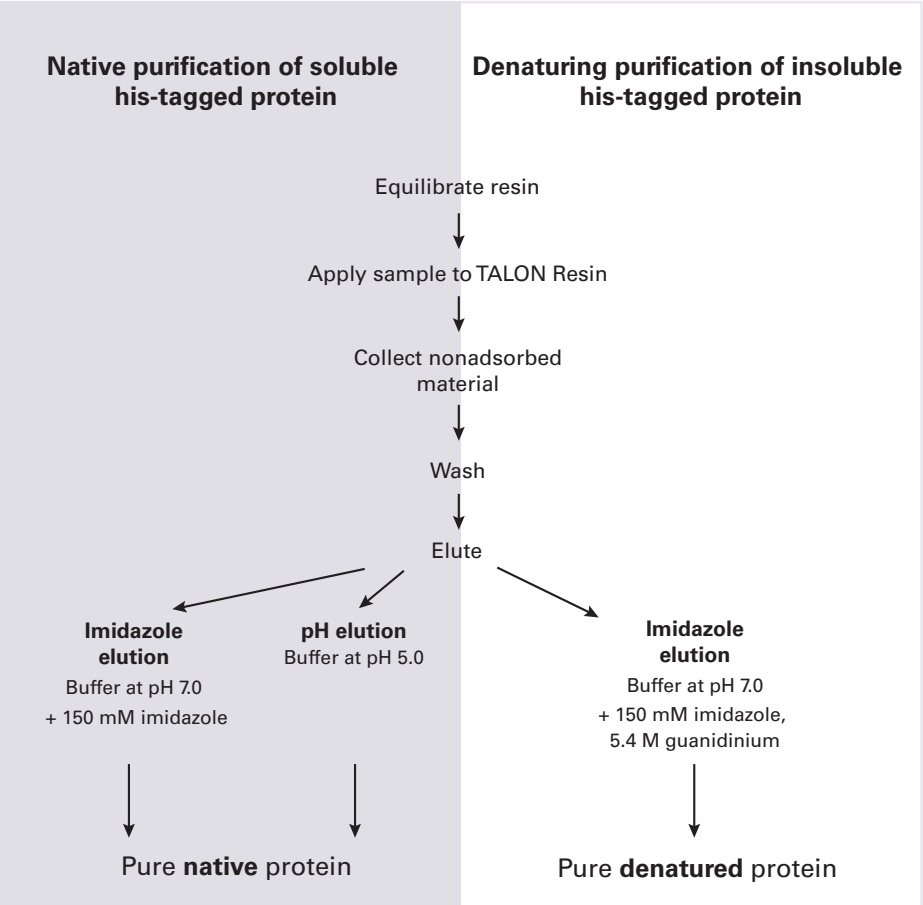


**Figure 1. Purification of his-tagged proteins using TALON Resin.** The protocols in this Protocol-at-a-Glance are designed using the Equilibration/Wash Buffer at pH 7.0. If your target protein is more stable at pH 8.0, or if it does not adsorb at pH 7.0, use the Equilibration Buffer (pH 8.0) instead of the Equilibration/Wash Buffer during the extraction and wash steps.

## C. Standard Sample Preparation to Isolate 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, below.
2. Resuspend the cell pellet by vortexing in 2 ml of chilled 1X Equilibration/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 Equilibration/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 (below). 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 30 sec pause 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 Cat. No. A-2039) for 2–3 min or until the composition of the mixture becomes paste-like. Add 2 ml chilled 1X Equilibration/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 Equilibration/Wash Buffer in order to inhibit metalloproteases during the extraction. Before application of the sample to the TALON Resin, EDTA must be removed by gel filtration chromatography (PD-10, GE Healthcare) equilibrated with the Equilibration/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. Reserve a small portion of the clarified sample at 4°C for SDS/PAGE analysis.
10. 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 small-scale purification (see Appendix B of the User Manual), and then analyze a portion by SDS/PAGE in parallel with protein standards. Once expression is observed, proceed with the purification protocol (see Section E).

**D. Standard 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** 1X Equilibration/Wash Buffer (pH 7.0) per 20–25 ml of culture.
3. Gently agitate 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 purification protocol (see Section E).

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

**E. His-Tagged Protein Purification**

1. Allow the column to stand at room temperature in an upright position until the resin to settles out of suspension.
2. Remove the column top cap and then the end cap, and allow the storage buffer to drain out until it is flush with the top of the resin bed.
3. Wash the column with 5 ml of distilled water.
4. Add 5 ml of Equilibration/Wash Buffer to equilibrate the column and allow the buffer to flow through.
5. Repeat Step 4.
6. Close the column with the end cap.
7. Add your clarified sample to the column.

   **Notes:**

   - We recommend maximum sample load of 8 mg of total protein over a single column. If loading higher amounts, additional washing steps should be performed.
   - Up to 5 ml of extract can be added to the column at a time. If your sample volume is larger than 5 ml, add the extract in steps.
8. Close the column with the top cap.
9. Gently agitate column with sample at room temperature for 20 min on a platform shaker to allow the his-tagged protein to bind to the column.

**Important:** The following steps can be performed at room temperature.

10. Let the column stand for 5 min in the upright position to allow the resin to settle out of suspension.
11. 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.
12. Wash column once with 5 bed volumes of 1X Equilibration/Wash Buffer.
13. **[Optional]:** If necessary, repeat Step 12 under more stringent conditions using 5–10 mM imidazole in 1X Equilibration/Wash Buffer (Section B.2).
14. Elute the his-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.
15. Use spectrophotometric and SDS/PAGE analyses to determine which fraction(s) contain(s) the bulk of the his-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, dialyze the fractions overnight against the Equilibration/Wash Buffer, and then measure their UV absorbance at 280 nm.

**Reference**

Sambrook, J. & Russell, D. W. (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

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