Capturem™ His-Tagged Purification Maxiprep Columns Protocol-At-A-Glance

I. Introduction

This protocol is provided for simple, rapid purification of his-tagged proteins from up to 25 ml of clarified lysate from mammalian or bacterial cell samples using the **Capturem His-Tagged Purification Maxiprep Columns** (Cat. Nos. 635715 & 635719). The columns are suitable for use under native or denaturing conditions, in the presence of additives such as DTT (up to 10 mM), βME (up to 30 mM), TCEP (up to 5 mM), EDTA (up to 10 mM), or glycerol glycerol (see reagent compatibility table for more information).

II. Materials and Reagents

A. Components

- 25 Capturem His-Tagged Purification Maxiprep Columns
  (spin columns with a clear insert and 50-ml collection tubes)

B. Additional Materials Required

1. Purification Buffers

- **xTractor™ Buffer** (for lysis and equilibration)
  (Cat. Nos. 635625, 635656, 635671 & 635623)
- **Wash Buffer** (20 mM Na_3PO_4, 150 mM NaCl, pH 7.6)
- **Elution Buffer** (20 mM Na_3PO_4, 500 mM NaCl, 500 mM imidazole, pH 7.6)

NOTE: We strongly recommend that you begin the purification procedure without using imidazole in your lysis and wash buffers when purifying standard proteins. We only suggest adding imidazole to the wash buffer if you notice significant background binding. xTractor Buffer does not contain imidazole.

2. Collection Tubes

Each purification will require four additional 50-ml collection tubes (standard conical disposable centrifuge tubes that can withstand centrifugation at up to 2,000*g*). We strongly recommend using a swinging bucket centrifuge for centrifuging Capturem His-Tagged Purification Maxiprep Columns, since using a fixed angle centrifuge may leave residual solution in the columns.

NOTE: When performing centrifugation, use the following formula to convert rpm to centrifugal force units (RCF or g) if the centrifuge does not automatically provide this information:

Centrifugal Force Conversion Formula: RCF or g = (1.12) x (R) x $(rpm/1{,}000)^2$

where R is the distance (in millimeters) from the center of the rotor to the end of the spin bucket when held horizontally and away from the center.

The 50-ml tubes should be used to collect cleared lysate, as well as flowthrough samples throughout the protocol, that will be saved for SDS-PAGE analysis and/or colorimetric protein assays (e.g., Bradford assays).

III. Sample Preparation

Before beginning the protein purification protocol in Section IV, it is necessary to prepare a cleared cell lysate from your bacterial or mammalian cell pellet. The lysate must be cleared by centrifugation before loading it onto a Capturem His-Tagged Purification Maxiprep Column (Section IV, Step 1). Lysis protocols using xTractor Buffer are provided in the xTractor Buffer & xTractor Buffer Kit User Manual. Individual protocols are also available for preparing cell lysates from bacterial, mammalian, baculovirus, and yeast cultures.

- **Bacterial Cell Samples**
  We recommend starting with a fresh or frozen cell pellet from 10–150 ml of overnight bacterial culture, which should yield 2–25 ml of cleared lysate.

  NOTE: When working with bacterial cells, the volume of lysate (containing the overexpressed his-tagged protein of interest) is determined by the amount of wet cell pellet obtained from a starting culture volume of 10–150 ml. For example, a log-phase *E. coli* culture (O.D. = 0.6–0.8), induced for 2–4 hr, would be expected to provide ~0.1–1.5 g of bacterial pellet from 10–150 ml of culture. We recommend adding ~2 ml of xTractor Buffer to each ~0.1 g of wet bacterial cell pellet.

- **Mammalian Cell Samples**
  We recommend starting with a fresh or frozen cell pellet from 10 ml of mammalian cell culture, (e.g., from from a 10-cm culture plate), which should be resuspended in 2 ml xTractor Buffer, yielding up to 2.5 ml of cleared lysate).
    - **For purification of intracellular his-tagged proteins:** Adherent cells may be harvested by treating them with trypsin and spinning them down, or scraping them directly from the well in the presence of xTractor Buffer. Suspension cells may be harvested by spinning down the liquid culture.
    - **For purification of secreted his-tagged proteins:** Cell culture supernatant can also be used to purify secreted proteins after it is cleared by centrifugation.

  NOTE: When lysing mammalian cells, you may substitute your standard lysis buffer for xTractor Buffer.

IV. His-Tagged Protein Purification

1. Add 6 ml xTractor Buffer to a Capturem His-Tagged Purification Maxiprep Column (clear insert) which has been placed in the provided collection tube, in order to equilibrate the column. Centrifuge at 2,000*g* for 3 min at room temperature. Remove the flowthrough and discard it along with the collection tube, then place the column in a new collection tube (supplied by the user—see Section II.B).
2. Load 2–25 ml cleared lysate (from Section III) onto the equilibrated column. Centrifuge at 2,000*g* for 3 min at room temperature. Save the collection tube containing the lysate flowthrough for protein analysis and transfer the column to a new collection tube (supplied by the user).

   NOTE: For proteins expressed at low levels, the flowthrough or additional filtered lysate (up to 25 ml) may be reloaded onto the column. However, we do not recommend reloading more than two times.

   If the solution does not fully drain from the column, perform a second centrifugation at 2,000*g* for 3 min. If the solution is still not draining completely, refer to Appendix A. Troubleshooting Guide and re-examine your lysate for viscosity, particles or cloudiness. For lysate preparation instructions, refer to the xTractor Buffer and xTractor Buffer Kit User Manual.

   Add 6 ml Wash Buffer to the column. Centrifuge at 2,000*g* for 3 min at room temperature. Save the collection tube containing the wash flowthrough for protein analysis and transfer the column to a new collection tube (supplied by the user).

   NOTE: Some purifications require optimization, and may benefit from addition of imidazole to the Wash Buffer. See Table 1, below, for instructions on how to prepare 10 ml of Wash Buffer containing different concentrations of imidazole (by combining different volumes of Wash Buffer and Elution Buffer).

Table 1. Adding Imidazole to Wash Buffer

Desired Imidazole Concentration	Wash Buffer Volume	Elution Buffer Volume
10 mM	9.8 ml	200 µl
20 mM	9.6 ml	400 µl
40 mM	9.2 ml	800 µl

3. Add 1.5 ml Elution Buffer to the column. Centrifuge at 2,000*g* for 3 min at room temperature. The collection tube should contain your eluted tagged protein, which is now ready for analysis.

   NOTE: 80–90% of your tagged protein can be eluted with 500–750 µl of Elution Buffer. When using a low elution volume, we recommend performing a second elution with the same volume of Elution Buffer in order to recover the remaining protein.

4. Measure the amount of protein in your flowthrough samples from Steps 2 and 3, and your eluate from Step 4, using a Bradford assay or other colorimetric protein analysis method.

5. Analyze the samples that were quantified in Step 5 using SDS-PAGE.

Appendix A. Troubleshooting Guide

Problem	Possible Explanation	Solution
Background bands/ low purity	Nonspecific binding of proteins to the membrane	• Add an additional wash step after binding with Wash Buffer. • Before loading the lysate in Section IV, include a blocking step between Steps 1 and 2 by adding BSA (100 µg) in a phosphate- or acetate-based buffer at pH 5 and spin at 2,000*g* for 3 min.
Low percentage recovery	The sample contains more his-tagged protein than the Capturem His-Tagged Purification Maxiprep Column has the capacity to bind.	Reduce the amount of sample added. If you need to purify more his-tagged protein, consider using Capturem His-Tagged Purification Large Volume, which has a higher binding capacity.
Low yield of his-tagged protein	Lysis Buffer contains imidazole, which interferes with his-tag binding.	Make sure that Lysis Buffer is free of imidazole. Our xTractor Buffer does not contain imidazole.
	Too much imidazole in Wash Buffer can elute his-tagged protein	Make sure the imidazole concentration in Wash Buffer is no higher than 40 mM.
His-tagged protein does not elute	Elution conditions are too mild, or elution buffer does not contain enough imidazole.	Follow the instructions using the recommended elution buffer containing the appropriate amount of imidazole.
Spin column does not fully drain	Clogging due to particles or a very viscous sample	• Prepare the lysate according to the xTractor Buffer and xTractor Buffer Kit User Manual. • If the lysate is not clear, centrifuge it a second time at 10,000–12,000*g* for 20 min or use a 0.45-micron filter (cellulose acetate) for further clarification. • Consider adding more DNase I to your lysate or lysozyme if appropriate (see xTractor Buffer and xTractor Buffer Kit User Manual). • Repeat Capturem His-Tagged Purification Maxiprep Column centrifugation at 2,000*g* for 3 min. If necessary, repeat this centrifugation one more time.

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tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
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