Capturem™ His-Tagged Purification Large Volume Protocol-At-A-Glance

I. Introduction

This protocol is provided for simple, rapid, room-temperature purification of his-tagged proteins in up to 500 ml of clarified lysate from mammalian or bacterial cell samples using **Capturem His-Tagged Purification Large Volume** (Cat. No. 635724) bottle-top units. Each unit requires a minimum elution volume of 1.5 ml. The units are suitable for use under native or denaturing conditions (see reagent compatibility table for more information).

II. Materials and Reagents

A. Components

- 4 Capturem His-Tagged Purification Large Volume Units (bottle-top units with white base)
- 1 Bottle Thread Adaptor (for a 33–45 mm bottle-neck diameter)

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 Bottles and Tubes

Each purification will require four ≥125-ml-sized (depending on sample volume) media bottles and two 50-ml collection tubes (standard conical disposable centrifuge tubes).

NOTE: Capturem His-Tagged Purification Large Volume bottle-top units are designed for 33-mm diameter thread bottles. A Bottle Thread Adaptor is included to ensure compatibility with 45-mm diameter thread bottles. Since the elution volumes from Capturem His-Tagged Purification Large Volume Units are small, we recommend using 50-ml tubes, which have a thread size of 33 mm in diameter, to collect the eluates.

Make sure to save a small aliquot of cleared lysate, as well as aliquots of flowthrough samples throughout the protocol, for SDS-PAGE analysis and/or colorimetric protein assays (e.g., Bradford assays).

3. Large Disposable Filtration Units

Each purification will require one or more large (150–500 ml) disposable filtration units (e.g., Nalgene Rapid-Flow Sterile Disposable Bottle Top Filters with PES Membrane [ThermoFisher Scientific, Cat. No. 295-3345], which have a capacity of 500 ml, or a similar filtration unit from another vendor).

4. Required Equipment

Purifications will require a vacuum source (e.g., a vacuum pump equipped with a trap and a vacuum controller, or a peristaltic pump).

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 and filtered via a large disposable filtration unit (supplied by the user—see Section II.B) before loading it into a Capturem His-Tagged Purification Large Volume Unit (Section IV, Step 3). 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 150–1,000 ml of overnight bacterial culture, which should yield ~25–200 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 150–1,000 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 ~1.5–10 g of bacterial pellet from 150–1,000 ml of culture. We recommend adding ~20 ml of xTractor Buffer to each ~1 g of wet bacterial cell pellet.

- **Mammalian Cell Samples**

  - **For purification of intracellular his-tagged proteins:**
    We recommend starting with fresh or frozen cell pellets from multiple 15-cm culture plates that yield 30 ml of mammalian cell culture each, up to a total of 500 ml of cell culture. Each pellet should be resuspended in 3 ml of xTractor Buffer, yielding up to 3.5 ml of cleared lysate. The volumes used in this extraction can be adjusted, provided that 20 µl of xTractor Buffer are used per 1 mg of cell pellet.

    NOTES:

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

  - **For purification of secreted his-tagged proteins:**
    Cell culture supernatant (up to 500 ml) can also be used to purify secreted proteins after it is cleared by centrifugation. For proteins expressed at low levels, an additional 500 ml of cleared cell culture supernatant may be loaded into the unit.

IV. His-Tagged Protein Purification

The following protocol describes how to purify his-tagged proteins from the cleared cell lysate prepared in Section III using a Capturem His-Tagged Purification Large Volume Unit.

IMPORTANT: When handling a Capturem His-Tagged Purification Large Volume Unit, make sure to hold it by the collar at the base of the unit, to avoid damaging the fragile upper container.

1. **Setting up the Capturem His-Tagged Purification Large Volume Unit**

    a. Assemble a Capturem His-Tagged Purification Large Volume Unit (see Appendix A), by placing it on top of a ≥125-ml-sized media collection bottle (supplied by the user; see Section II.B.2) and securing the connection.

    b. Make sure the connection between the unit and the bottle is not overtightened, and then connect the tubing from the vacuum source to the vacuum outlet on the purification unit.

    NOTE: You may need to reinforce the connection with Parafilm to ensure an even flow and prevent bubble formation due to air escaping between the unit, the adaptor (if using a 45-mm diameter thread collection bottle), and the collection bottle/tube.

2. **Equilibrating the Capturem His-Tagged Purification Large Volume Unit**

    a. Pipet 20 ml of xTractor Buffer into the purification unit and apply a vacuum to pull the buffer through the unit. The buffer should flow out of the unit as a steady stream of liquid without bubbles.

    b. After all of the xTractor buffer has passed through the purification unit (the membrane should appear to be dry), turn off the vacuum, and disconnect the tube from the unit. Remove the collection bottle and replace it with a clean ≥125-ml-sized collection bottle labeled FT (supplied by the user; see Section II.B.2).

    NOTE: The vacuum may need to be adjusted using the vacuum controller to obtain optimal flow rates. Usually the flow rate will vary between 5–50 ml/min, depending on the viscosity of the solution added to the purification unit.

3. **Loading the Capturem His-Tagged Purification Large Volume Unit**

    a. Pipet 25–500 ml of cleared lysate (from Section III) into the equilibrated Capturem His-Tagged Purification Large Volume Unit. Connect the vacuum tubing to the purification unit and apply a vacuum to pull the lysate through the unit.

    b. After all of the lysate has passed through the unit, turn off the vacuum and disconnect the tubing from the unit. Save the collection bottle containing the lysate flowthrough for protein analysis and replace it with a clean ≥125-ml-sized collection bottle labeled W for Wash (supplied by the user; see Section II.B.2).

    NOTE: For proteins expressed at low levels, the flowthrough or additional filtered lysate (up to 500 ml) may be reloaded into the unit. However, we do not recommend reloading more than two times.

4. Washing the Capturem His-Tagged Purification Large Volume Unit

a. Pipet 30–50 ml of Wash Buffer into the Capturem His-Tagged Purification Large Volume Unit. Make sure to run the Wash Buffer down the sides of the filtration unit to rinse off any remaining lysate. Connect the vacuum tubing to the purification unit and apply a vacuum to pull the Wash Buffer through the unit.

b. After all of the buffer has passed through the unit, turn off the vacuum and disconnect the tubing from the unit. Save the collection bottle containing the flowthrough for protein analysis and replace with a clean 50-ml collection tube labeled E for Elution fraction (supplied by the user; see Section II.B.2). Make sure to place this tube in a rack where it is stable and level to the benchtop, so the purified protein will elute evenly from the membrane of the unit in Step 5.

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 100 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	98 ml	2 ml
20 mM	96 ml	4 ml
40 mM	92 ml	8 ml

5. Eluting the Capturem His-Tagged Purification Large Volume Unit

a. Pipet 20 ml of Elution Buffer into the Capturem His-Tagged Purification Large Volume Unit. Connect the vacuum tubing to the purification unit and apply a vacuum to pull the Elution Buffer through the unit.

b. After all of the buffer has passed through the unit, turn off the vacuum and disconnect the tubing from the unit. 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 7.5–10 ml of Elution Buffer. When using a low elution volume, we recommend performing a second elution with the same volume of Elution Buffer to recover the remaining protein.

6. Analysis

a. Measure the amount of protein in your eluate(s) from Step 5.b, using a Bradford assay or other colorimetric protein analysis method.

b. Analyze the flowthrough samples from Steps 3.b and 4.b, and your eluate sample(s) that were quantified in Step 6.a, using SDS-PAGE.

Appendix A. Setting up the Purification Unit

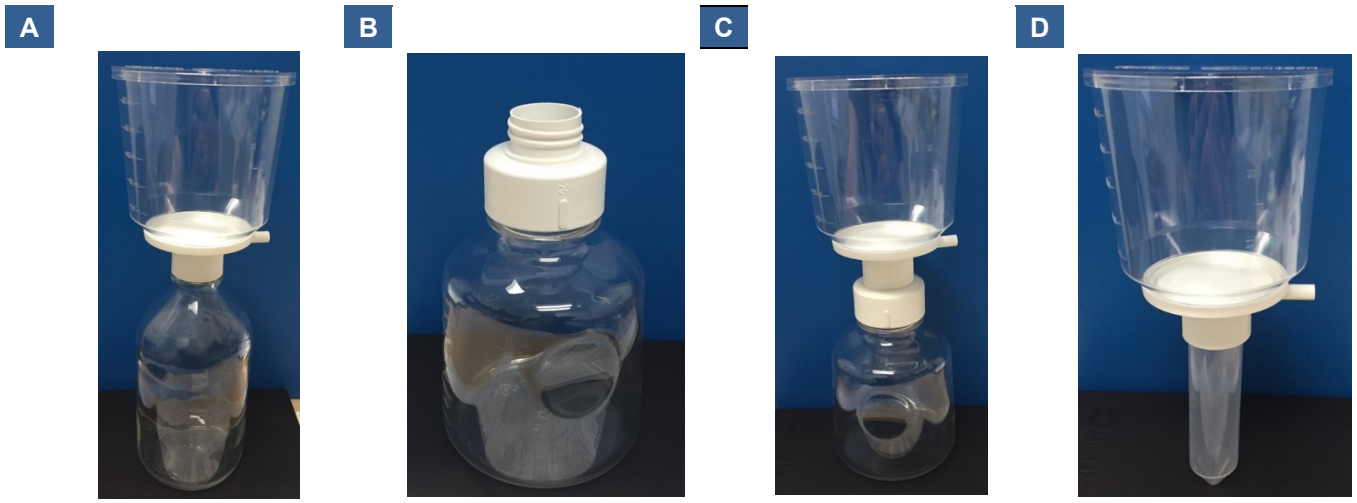

Figure 1. Setting up the Capturem His-Tagged Purification Large Volume Unit. The Capturem His-Tagged Purification Large Volume Unit connects directly to a 33-mm diameter thread bottle (**Panel A**). The provided Bottle Thread Adaptor (**Panel B**) allows the purification unit to connect to a 45-mm diameter thread bottle (**Panel C**). The purification unit connects directly to a 50-ml tube for elution (**Panel D**).

Appendix B: Example Experiment

In an example experiment, the Capturem His-Tagged Purification Large Volume Unit was used to purify overexpressed 6xHis-GFPuv from a bacterial cell lysate (Figure 2).

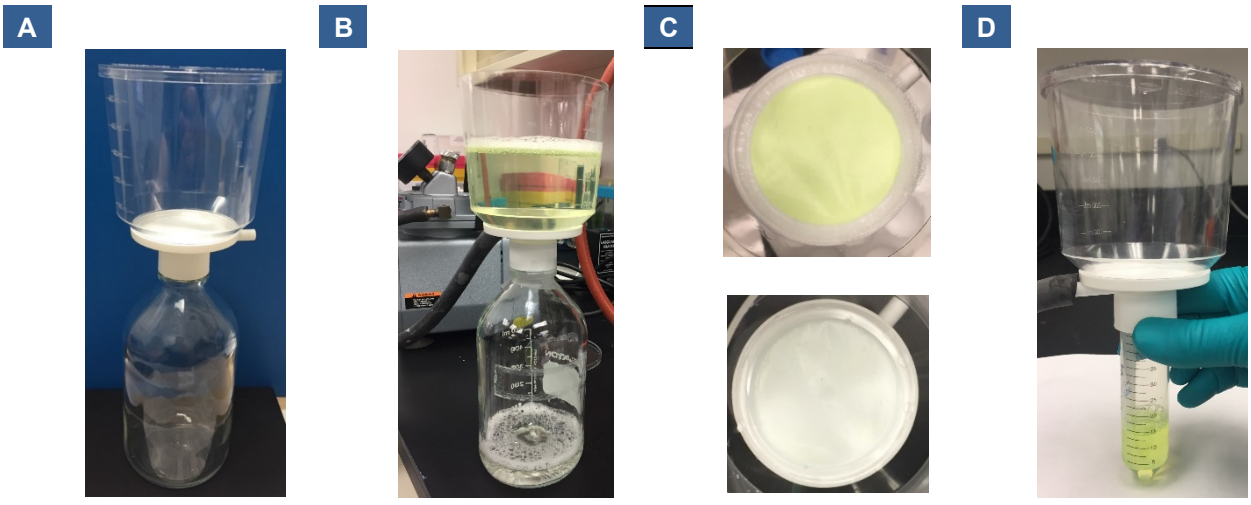

Figure 2. Using the Capturem His-Tagged Purification Large Volume Unit. **Panel A.** A Capturem His-Tagged Purification Large Volume Unit is connected to a 33-mm diameter thread bottle. **Panel B.** A clarified lysate containing overexpressed 6xHis-GFPuv is loaded into the unit. **Panel C.** The unit membrane retains the characteristic yellowish green color of green fluorescent protein (GFP) after the loading and washing steps (top), and returns to its original white color after the 6xHis-GFPuv is eluted (bottom). **Panel D.** The 6xHis-GFPuv is eluted into a 50-ml collection tube.

Appendix C. 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 1 min.
Low percentage recovery	The sample contains more his-tagged protein than the Capturem His-Tagged Purification Large Volume Unit 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.

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