I. Introduction

This protocol is provided for the **Capturem His-Tagged Purification 24-Well Plate** (Cat. No. 635730), a single-use disposable 24-well plate that allows simple, rapid purification of his-tagged proteins expressed in mammalian or bacterial cell samples from up to 4 ml of clarified lysate per well, using either vacuum filtration or centrifugation. Each well requires a minimum elution volume of 500 µl. This plate is suitable for use under native or denaturing conditions (see reagent compatibility table for more information).

II. Materials and Reagents

A. Components

- 1 Capturem His-Tagged Purification 24-Well Plate

B. Additional Materials Required

1. Purification Buffers

- **Lysis Buffer:** We recommend using xTractor™ Buffer (Cat. Nos. 635625, 635656, or 635671), but you may substitute your standard lysis buffer. 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.
- **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

2. Collection Plates

- **24-well collection plates:** Each purification will require four standard 24-well collection plates (e.g., E&K Scientific, Cat. No. EK-2239, 24-well storage plate, square top 10 ml, deep well, round bottom). These plates should be used throughout the protocol to collect the flowthrough and wash samples that will be saved for SDS-PAGE analysis and/or colorimetric protein assays (e.g., Bradford assays). Alternatively, if only the eluates are to be analyzed, the flowthrough from the equilibration and wash steps can be discarded. In this case, only two collection plates are required, one for collecting the flowthrough and washes and a second for the actual eluates.
- **Recommended filtration device:** The Capturem His-Tagged Purification 24-Well Plate is compatible with standard 96-well plate filtration devices, such as the NucleoVac 96 Vacuum Manifold (Cat. No. 740681).
- **Recommended plate centrifuge:** The Capturem His-Tagged Purification 24-Well Plate is compatible with standard 96-well plate centrifuges (e.g., Eppendorf Centrifuge 5804 R with Deepwell-plate rotor, A-2-DWP).
- **Single-channel pipettes** (various)

III. Sample Preparation

Express your his-tagged protein(s) of interest in 1 ml cultures grown in 24-well plates or 2–5-ml cultures grown in 6-well plates. Before beginning the protein purification protocol in Section IV, it is necessary to prepare crude cell lysates from each culture and clarify the lysates by centrifugation or filtration. We recommend using xTractor Buffer (Section II) for lysis. 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 2–5 ml of overnight bacterial culture, which should yield 200–1,000 µl 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 2–5 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 ~20–80 mg of bacterial pellet from 2–5 ml of culture. We recommend adding ~400 µl of xTractor Buffer to each ~20 mg of wet bacterial cell pellet.

- **Mammalian Cell Samples**

  For purification of intracellular his-tagged proteins:
  We recommend starting with a fresh or frozen cell pellet from 2 ml of mammalian cell culture, (e.g., from a single well of a 6-well culture plate), which should be resuspended in 100–500 µl of xTractor Buffer, yielding up to 600 µl 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. 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.

  NOTE: 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 4.5 ml) can also be used to purify secreted proteins after it is clarified by centrifugation. For proteins expressed at low levels, an additional 4.5 ml of cleared cell culture supernatant may be loaded into the unit.

IV. His-Tagged Protein Purification

Purification of his-tagged proteins from the clarified cell lysates prepared in Section III may be performed using vacuum filtration (Protocol A) or centrifugation in a standard 96-well plate centrifuge (Protocol B).

A. Protocol: Protein Purification Using Vacuum Filtration

1. Assemble the Capturem His-Tagged Purification 24-Well Plate in a filtration device together with a 24-well collection plate (supplied by the user—see Section II.B).
2. Add 2 ml of Lysis Buffer or PBS to each well of the Capturem His-Tagged Purification 24-Well Plate to equilibrate the wells. Use a vacuum (~0.3–0.4 bar) to run the buffer through the Capturem plate wells into the collection plate. Remove the flowthrough and discard it along with the collection plate. Reassemble the Capturem His-Tagged Purification 24-Well Plate in the filtration device together with a new collection plate.

   NOTE: The required pressure may vary between 0.2–0.8 bar, depending on the sample. When performing vacuum filtration, use the following conversion factors to convert between different vacuum pressure units:

   Pressure conversion factors:
   1 mbar = 100 Pa = 0.750 mm Hg = 14.504 x 10^{-3} psi = 0.987 x 10^{-3} atm
3. Load 0.5–4.5 ml of cleared lysate (from Section III) into each well of the equilibrated Capturem His-Tagged Purification 24-Well Plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the Capturem plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis. Reassemble the Capturem His-Tagged Purification 24-Well Plate in the filtration device together with a new collection plate.
4. Add 2 ml of Wash Buffer to each well of the Capturem His-Tagged Purification 24-Well Plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the Capturem plate wells into the collection plate. Save the collection plate containing the wash flowthrough for protein analysis.

   NOTE: Some purifications require optimization and may benefit from addition of imidazole to the Wash Buffer. See Table I, below, for instructions on how to prepare 100 ml of Wash Buffer containing different concentrations of imidazole (by combining different volumes of Wash Buffer).

   Table I. 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. Add 1 ml of Elution Buffer to each well of the Capturem His-Tagged Purification 24-Well Plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the Capturem plate wells into the collection plate. The wells of the collection plate should contain your eluted protein, which is now ready for analysis.

   NOTE: ≥90% of your tagged protein can be eluted with 500 µl of Elution Buffer.

6. Measure the amount of protein in your eluates from Step 5 using a Bradford assay or other colorimetric protein analysis method.
7. Analyze the flowthrough samples from Steps 3 and 4, and your eluate samples that were quantified in Step 6, using SDS-PAGE.

B. Protocol: Protein Purification Using Centrifugation

1. Place the Capturem His-Tagged Purification 24-Well Plate securely on top of a 24-well collection plate (supplied by the user—see Section II.B).
2. Add 2 ml of Equilibration Buffer to each well of the Capturem His-Tagged Purification 24-Well Plate to equilibrate the wells. Centrifuge at 600*g* for 2 min at room temperature. Remove the flowthrough and discard it along with the collection plate. Place the Capturem His-Tagged Purification 24-Well Plate securely on top of a new 24-well collection plate.

   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 $g = (1.12) \times (R) \times (rpm/1{,}000)^2$

   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.

   If the solution does not fully drain from the column, perform a second centrifugation at 600*g* for 2 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.
3. Load 0.5–4.5 ml of cleared lysate (from Section III) into each well of the equilibrated Capturem His-Tagged Purification 24-Well Plate. Centrifuge at 600*g* for 2 min at room temperature. Save the collection plate containing the flowthrough for protein analysis. Place the Capturem His-Tagged Purification 24-Well Plate securely on top of a new 24-well collection plate.
4. Add 2 ml of Wash Buffer to each well of the Capturem His-Tagged Purification 24-Well Plate. Centrifuge at 600*g* for 2 min at room temperature. Save the collection plate containing the flowthrough for protein analysis. Place the Capturem His-Tagged Purification 24-Well Plate securely on top of a new 24-well collection plate.

   NOTE: Some purifications require optimization and may benefit from addition of imidazole to the Wash Buffer. See Table I, above, for instructions on how to prepare 100 ml of Wash Buffer containing different concentrations of imidazole (by combining different volumes of Wash Buffer).
5. Add 1 ml of Elution Buffer to each well of the Capturem His-Tagged Purification 24-Well Plate. Centrifuge at 600*g* for 2 min at room temperature. The wells of the collection plate should contain your eluted tagged protein, which is now ready for analysis.

   NOTE: ≥90% of your tagged protein can be eluted with 500 µl of Elution Buffer.
6. Measure the amount of protein in your eluates from Step 5 using a Bradford assay or other colorimetric protein analysis method.
7. Analyze the flowthrough samples from Steps 3 and 4, and your eluate samples that were quantified in Step 6, 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.A or IV.B, 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 600*g* for 2 min.
Low percentage recovery	The sample contains more his-tagged protein than the Capturem His-Tagged Purification 24-Well Plate has the capacity to bind.	Reduce the amount of sample added to each well. If you need to purify more his-tagged protein, consider using the Capturem His-Tagged Purification Maxiprep Kit or Capturem His-Tagged Purification Large Volume, which have higher binding capacities.
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 appropriate amount of imidazole.
Wells do 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 24-Well Plate centrifugation at 600*g* for 2 min. If necessary, repeat this centrifugation one more time.

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