TECH NOTE

Rapid, high-purity isolation of his-tagged proteins directly from large volumes of cell lysate and culture supernatant

Capturem His-Tagged Purification Large Volume

Purify concentrated protein from large sample volumes: 50 ml to >500 ml (at a time) >>

Obtain highly pure protein in record time: 10–30 min >>

Simplify purification of poorly expressed proteins, such as secreted proteins >>

Introduction

Efficient purification of recombinant proteins is a vital procedure in both academic and industrial research settings. Protein fusion tags facilitate the production of enough pure protein to effectively study protein structure and function. Proteins linked to polyhistidine tags (e.g., 6xhis tags), the most popular fusion tag, are commonly purified using resin-based immobilized metal affinity chromatography (IMAC) columns. IMAC chromatography can be quite time-consuming, requiring several hours to complete, thus exposing proteins to possible protease degradation. Capturem his-tagged purification kits provide an alternative purification method that uses next-generation membrane technology to quickly and efficiently purify and concentrate the target protein in one step, at room temperature.

Poorly expressed and secreted proteins present a special purification challenge, since it may be necessary to purify them from large volumes of cell lysate or culture medium. Therefore, in addition to the miniprep, maxiprep, and 96-well plate formats, Capturem technology is now available in the Capturem His-Tagged Purification Large Volume format—single-use, disposable bottle-top units for use with up to 500 ml (at a time) of cleared lysate or medium. A Capturem His-Tagged Purification Large Volume Unit (Figure 1) connects directly to a 33-mm diameter bottle (Figure 1, Panel A) and is also compatible with a 45-mm bottle using the provided Bottle Thread Adaptor (Figure 1, Panels B and C). The unit's membrane effectively concentrates the purified protein so it can be eluted into a 50-ml tube which connects directly to the unit (Figure 1, Panel D).

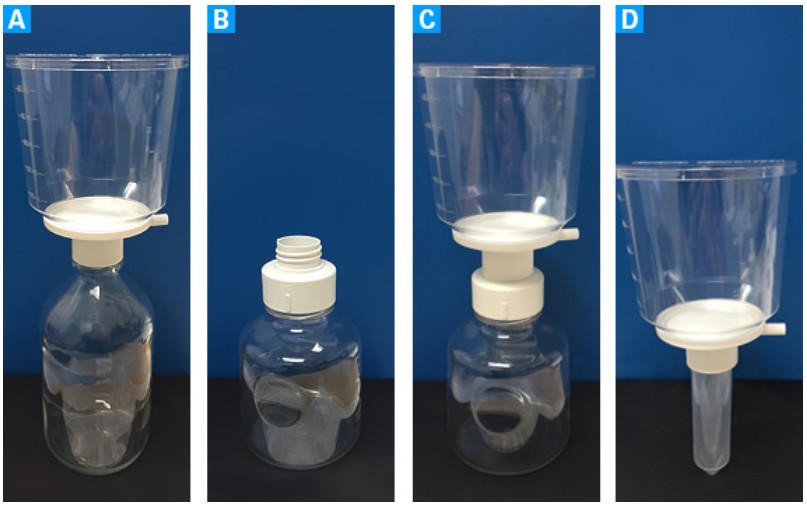

Figure 1. Setting up the Capturem His-Tagged Purification Large Volume Unit. The Capturem His-Tagged Purification Large Volume Unit connects directly to a 33-mm diameter thread glass bottle (**Panel A**). The provided Bottle Thread Adaptor (**Panel B**) allows the purification unit to connect to a 45-mm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

To illustrate the simplicity of the Capturem His-Tagged Purification Large Volume procedure, 6xhis-GFPuv was purified using a Capturem His-Tagged Purification Large Volume Unit (Figure 2). The 6xhis-GFPuv bound to the membrane during loading steps (Figure 2, Panel B and Panel C, top) and this yellowish-green protein was released during the elution step (Figure 2, Panel C, bottom and Panel D).

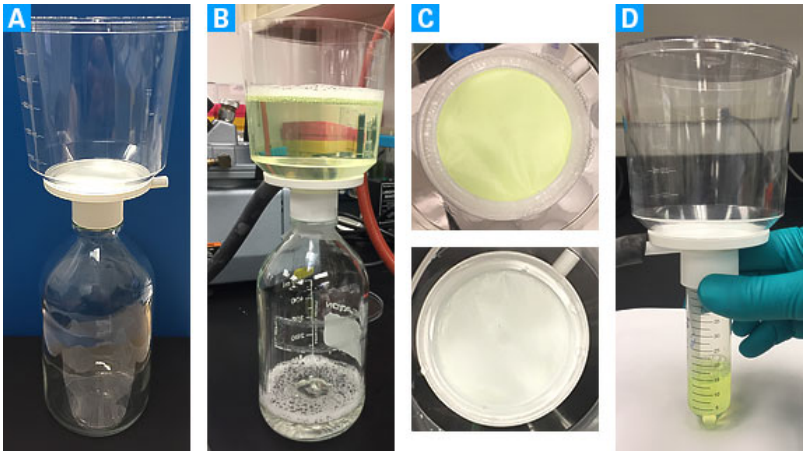

Figure 2. Using the Capturem His-Tagged Purification Large Volume Unit. 6xhis-GFPuv was purified using a Capturem His-Tagged Purification Large Volume Unit as described in the Methods section below. **Panel A.** A Capturem His-Tagged Purification Large Volume Unit is connected to a 33-mm diameter thread glass bottle. **Panel B.** A clarified lysate containing overexpressed 6xhis-GFPuv is loaded into the unit. **Panel C.** The membrane retained 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.

Results

Highly pure protein from a large volume of lysate

A Capturem His-Tagged Purification Large Volume Unit was used to purify 27 mg of his-tagged GFPuv (6xhis-GFPuv) from 100 ml of clarified cell lysate in an elution volume of 15 ml. The entire purification was completed in just 15 min. Analysis of the cleared lysate, flowthrough, and eluate using SDS-PAGE shows effective purification of a ~29-kDa molecular weight band corresponding to 6xhis-GFPuv (Figure 3).

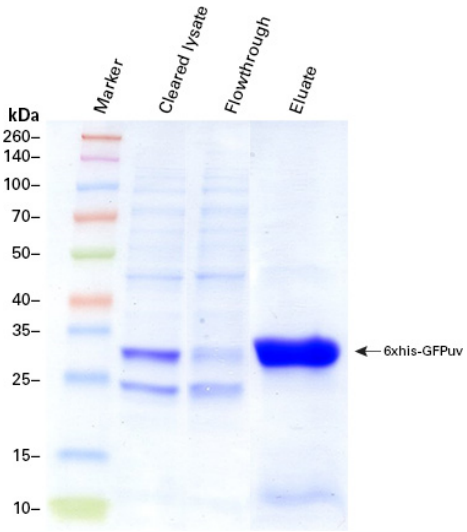

Figure 3. Purification of 6xhis-GFPuv using a Capturem His-Tagged Purification Large Volume Unit. A Capturem His-Tagged Purification Large Volume Unit was used to purify 6xhis-GFPuv from 100 ml of cleared cell lysate. The lysate, flowthrough, and eluate were analyzed using SDS-PAGE. Protein purification and SDS-PAGE were performed as described in the Methods section below.

Highly pure protein in record time

A Capturem His-Tagged Purification Large Volume Unit was used to purify 10 mg of his-tagged mCherry (6xHN-mCherry) from 200 ml of clarified cell lysate in an elution volume of 9 ml. The entire purification was completed in about 10 min. Analysis of the crude lysate, cleared lysate, flowthrough, and eluate using SDS-PAGE shows effective purification of a ~30-kDa molecular weight band corresponding to 6xHN-mCherry (Figure 4). The unit yielded highly pure protein even though it was intentionally overloaded.

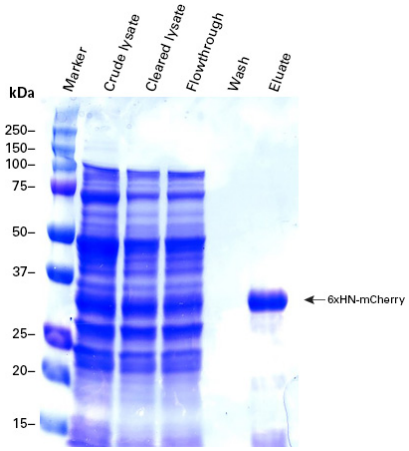

Figure 4. Purification of 6xHN-mCherry using a Capturem His-Tagged Purification Large Volume Unit. A Capturem His-Tagged Purification Large Volume Unit was used to purify 6xHN-mCherry from a cleared lysate derived from 6 g of cell pellet. The crude and cleared lysates, as well as the flowthrough, wash, and eluate, were analyzed using SDS-PAGE. Protein purification and SDS-PAGE were performed as described in the Methods section below.

Simplified purification of active secreted proteins

Purification of secreted proteins can be especially challenging because such proteins may need to be purified from large volumes of culture supernatant when concentrations are so low as to be undetectable by Western blotting. A Capturem His-Tagged Purification Large Volume Unit was used to purify his-tagged *Metridia* luciferase (6xhis-MetLuc) from 1.2 L of clarified cell supernatant in an elution volume of 7.5 ml. The entire purification was completed in less than 30 min (Figure 5).

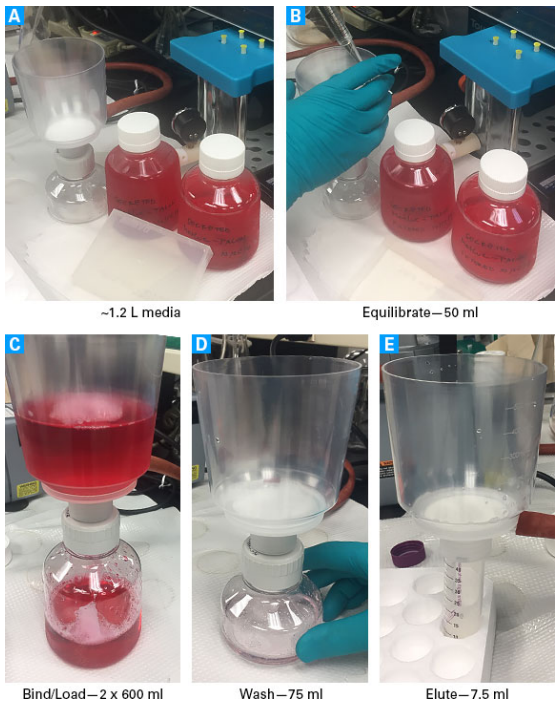

Figure 5. Purification of secreted his-tagged *Metridia* luciferase. A Capturem His-Tagged Purification Large Volume Unit was used to purify his-tagged *Metridia* luciferase (6xhis-MetLuc) from 1.2 L of clarified cell supernatant collected from a HEK293 CMV-MetLuc stable cell line (**Panel A**) line as described in the Methods section below. The unit was equilibrated with 50 ml of xTractor Buffer (**Panel B**) and loaded twice with 600 ml of cell culture supernatant for a total of 1.2 L (**Panel C**). The unit was washed with 75 ml of Wash Buffer (**Panel D**) and eluted with 7.5 ml of Elution Buffer (**Panel E**). The entire purification was completed in less than 30 min.

Western blot analysis of an SDS-PAGE gel containing samples of cleared lysate, flowthrough, and eluate with anti-MetLuc and anti-6xhis antibodies showed effective purification of a ~24-kDa molecular weight band corresponding to 6xhis-MetLuc (Figure 6). This poorly expressed protein has been concentrated by purification with a Capturem His-Tagged Purification Large Volume Unit, so it is now visible on Western blots, unlike the original sample. The purified 6xhis-MetLuc was shown to be active using a Ready-To-Glow Secreted Luciferase Reporter Assay (data not shown).

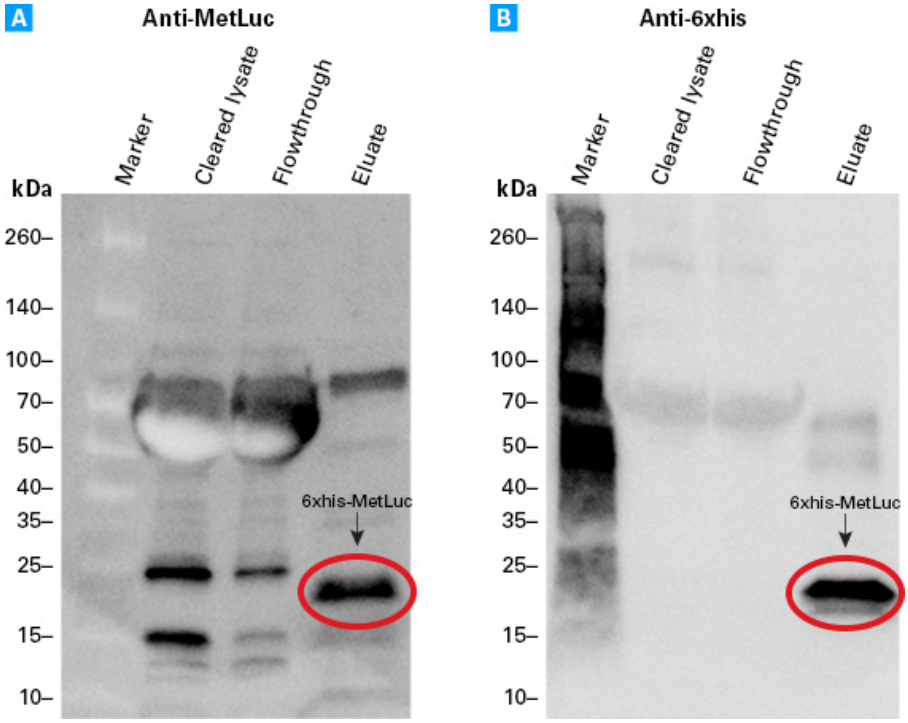

Figure 6. Western blot analysis of secreted, his-tagged *Metridia* luciferase purified using a Capturem His-Tagged Purification Large Volume Unit. 6xhis-MetLuc was purified using a Capturem His-Tagged Purification Large Volume Unit as described in the Methods section below. The cleared lysate, flowthrough, and eluate were analyzed using SDS-PAGE, transferred to a PVDF membrane for Western blotting, and probed with rabbit polyclonal anti-MetLuc antibody (**Panel A**) and mouse monoclonal anti-6xhis antibody (**Panel B**). Protein purification, SDS-PAGE, and Western blotting were performed as described in the Methods section below.

Conclusions

Capturem His-Tagged Purification Large Volume can be used to obtain highly pure, concentrated protein from large volumes of clarified cell lysate using a simple, rapid protocol. This new technology yields protein of high purity, even when the unit is overloaded. The units are also effective at purifying and concentrating active secreted protein from large volumes of cell culture supernatant. These features make Capturem His-Tagged Purification Large Volume a powerful tool for isolating secreted proteins or proteins that are poorly expressed, in less than 30 min, in amounts that are sufficient for protein characterization studies and downstream applications.

Methods

6xhis-GFPuv was purified with a Capturem His-Tagged Purification Large Volume Unit, using a vacuum system. Protein was extracted from 5 g of cell pellet with xTractor Buffer (200 ml) and then filtered to clarify the lysate. The unit was equilibrated with 20 ml of xTractor Buffer and loaded with 100 ml of the filtered lysate. After a 30 ml wash, purified protein was eluted in a volume of 15 ml, using the wash and elution buffers described in the Capturem His-Tagged Purification Large Volume Protocol-At-A-Glance. The protein concentration was measured using a NanoDrop spectrophotometer, and the clarified lysate, flowthrough, and eluate were analyzed using a 4–20% SDS-PAGE gel.

6xhis-mCherry was purified with a Capturem His-Tagged Purification Large Volume Unit, using a vacuum system. Protein was extracted from 6 g of cell pellet with xTractor Buffer (200 ml) and then filtered to clarify the lysate. The unit was equilibrated with 20 ml of xTractor Buffer and loaded with 100 ml of the filtered lysate. After a 30 ml wash, purified protein was eluted in a volume of 9 ml. The protein concentration was measured using a NanoDrop spectrophotometer, and the crude lysate, clarified lysate, flowthrough, and eluate were analyzed using a 4–20% SDS-PAGE gel.

6xhis-MetLuc was purified with a Capturem His-Tagged Purification Large Volume Unit, using a vacuum system. Protein was extracted from 1.2 L of cell culture supernatant that was collected from a HEK293 CMV-MetLuc stable cell line (from a total of 35 x 15 cm plates), and clarified by centrifugation. The unit was equilibrated with 50 ml of PBS and loaded with the entire filtered lysate, at a flow rate of 50 ml/min. After a 75 ml wash, purified protein was eluted in a volume of 7.5 ml. The protein concentration was measured using a NanoDrop spectrophotometer, and the clarified lysate, flowthrough, and eluate were analyzed using a 4–20% SDS-PAGE gel, which was transferred to a PVDF membrane for Western blotting. Two separate blots containing the same samples were probed with either a primary rabbit polyclonal anti-MetLuc antibody (1:500 dilution) or a primary mouse monoclonal anti-6xhis antibody (1:1,000 dilution), each followed by a secondary antibody (1:5,000 dilution), and detection with Femto Detection Reagent. The fluorescence activity of the purified 6xhis-MetLuc was measured using a Ready-To-Glow Secreted Luciferase Reporter Assay (data not shown).

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