Purification of High-Expressed Proteins

Introduction

Recombinant proteins are widely used in life sciences, biotechnology, and medicine, and there is a growing need for innovative technologies that enable their rapid and cost-effective purification. Some of the most successful protein purification schemes make use of affinity tags that are genetically fused to the protein of interest (POI).[1] A number of such tags have been developed, and include the broadly-used hexahistidine (6xHis) tag that binds to Ni^{2+}-nitrilotriacetic acid (NTA) resin.[2] However, Ni^{2+}-NTA and other commonly used affinity purification resins contain toxic metal ions or reagents that inactivate certain proteins and preclude their use for *in vitro* studies.[3,4,5] In addition to that, the most significant disadvantages to the use of His tags are their high cost and lack of scalability when advancing to manufacturing.

A new affinity tag has been developed which meets or exceeds the specifications of His tags in terms of performance – including a significant decrease in cost and the ability to scale from research to preparative to manufacturing. Recombinant high-expressed proteins can now be purified in less than 30 minutes from *E. coli* BL21(DE3) host cells with a final purity greater than 98%, a final yield up to 8mg, and a concentration up to 1mg/mL without the use of metal ions or toxic reagents.

Three recombinant proteins were expressed and purified using this new affinity tag to demonstrate the applicability of this method for the purification of high-expressed proteins (HEP).

GLK: *Thermus thermophilus* glucokinase is a homo-tetrameric protein. Oligomeric proteins can be difficult to purify because affinity tags may be sterically blocked from interacting with the resin. GLK is usually expressed at high levels in bacteria host cells.

MBP: maltose-binding protein is usually very highly expressed in bacteria host cell and it is used as solubilizing partner for insoluble recombinant protein.

sfGFP: Superfolder GFP is green fluorescent protein derived from *Aequorea victoria*. It is generally used as reporter protein.

We demonstrate the purification of these three proteins using the Proteios Car9 affinity tag and the Proteios HEP Purification Kit. Proteios Car9 is a dodecapeptide with high affinity for silica substrates and whose binding is competitively inhibited by L-Lysine.[6,7] This forms the basis of Proteios' recombinant protein purification technology.

Materials and Methods

Cell Lines

BL21(DE3) (One Shot™ BL21(DE3) Chemically Competent *E. coli*) and Top10 cells (GeneArt™ Gibson Assembly HiFi Cloning Kit, chemically competent cells) were purchased from Thermo Scientific. Plasmid extraction kits were purchased from Thermo Scientific.

Cloning, Cell Growth, Protein Expression and Cell Collection

A dodecapeptide coding for the Proteios Car9 sequence (DSARGFKKPGKR) was introduced either at the N-terminal or at the C-terminal of the POI by PCR. A six amino acid spacer (KLGGGS) was placed between the Proteios Car9 tag and the POI to minimize the effect of the tag. The modified CoDing Sequence (CDS) was introduced into pET24+ plasmid by enzymatic digestion with NdeI and XhoI. Plasmids were amplified in the Top10 bacteria host. BL21(DE3) bacteria strain was used to express the desired protein at 37˚C for 3–4 hours under shaking at 200rpm. Protein expression was induced with 1mM IPTG or Arabinose 0.1%.

Bacteria cells were collected by centrifugation at 3500g for 10 minutes at 4˚C.

Cell Lysis

Cell pellets from 50mL cultures were destroyed either by three cycles of freeze–thaw or by six cycles of sonication (5sec on, 30sec off, 30% power, in ice) in 4mL of Proteios HEP Lysis Buffer supplemented with 1mM PMSF and 2mM EDTA. Cellular debris was removed at 10000g for 15min at 4˚C. Clarified cell lysate was immediately used or stored at -20ºC for future use.

Protein Purification

A Proteios HEP Purification Kit was used to purify proteins that express from high to very high levels according to the manufacturing instructions.

Briefly, the provided gravity columns were first pre-washed with 6ml of Proteios HEP Wash Buffer. Up to 8mL of clarified lysates were loaded on the column and incubated at room temperature for 5 minutes. The lysate was allowed to drain through the column. The flow-through was discarded and the gravity column was washed three times with 8mL of Proteios HEP Wash Buffer. The protein of interest was eluted in a 50mL collection tube using 4mL Proteios HEP Elution Buffer. Another additional elution was performed to elute the remaining protein.

Protein Desalting

Buffer exchange was performed using dialysis tubing (SnakeSkin™ Dialysis Tubing, 3.5K MWCO, 16 mm) with a cutoff of 3.5kDa against 20mM Tris pH 8 overnight at 4ºC or using centrifugal filter units.

Purity and Yield Determination

Purity was determined by SDS-PAGE followed by PageBlue protein staining solution (PageBlue™ Protein Staining Solution) and image analysis using ImageJ analysis software. Yield was determined using the Thermo Scientific™ NanoDrop™ OneC Microvolume UV-Vis Spectrophotometer through absorption at 280nm.

Results

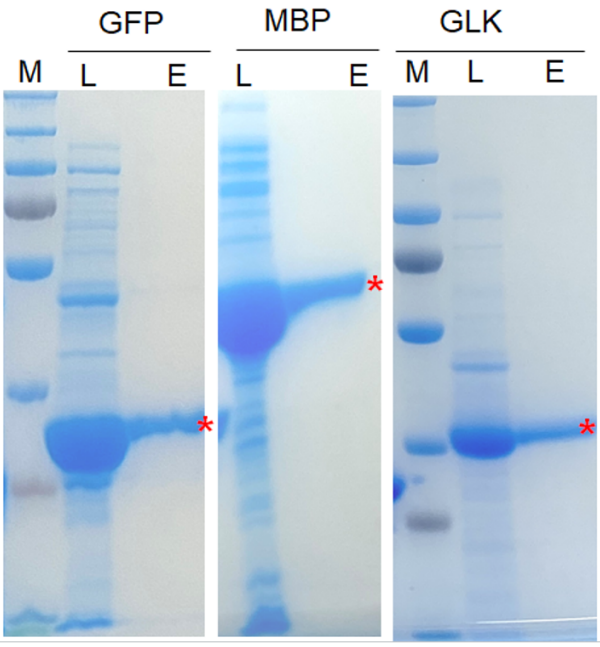

Figure 1: Purification of protein of interest using Proteios HEP Purification Kit for High-expressed protein. sfGFP-Car9 (left), MBP-Car9(middle) and Car9-GLK (right) are indicated with '*'. M: Protein ladder (PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa), L: Lysate, E: Elution.

The Proteios HEP Purification Kit was able to purify up to 8mg of protein using a single column (**Figure 1**). A purity > 98% and a POI concentration in the elute up to 1mg/mL was achieved without the use of toxic metal ions or toxic reagents (**Table 1**). The protein purification step took less than 30 minutes in total.

Protein	Level of Expression in Lysate	Purity	Yield	Concentration
sfGFP	Very High	98%	7.8mg/50mL culture	1mg/mL
MBP	Very High	99%	8.0mg/50mL culture	1mg/mL
GLK	High	99%	2.0mg/50mL culture	0.25mg/mL

Table 1: Purity and yield obtained using Proteios HEP Purification Kit.

Conclusion

Proteios HEP Purification Kit provides a quick, low-cost alternative to His tag technology to purify up to 8mg of protein that expresses from high to very high levels in less than 30 minutes with an average purity > 98% and with a POI concentration in the elution fraction up to 1mg/mL without the use of toxic metal ions or toxic reagents.

References

1. Zhao X, Li G, Liang S. Several affinity tags commonly used in chromatographic purification. J Anal Methods Chem. 2013;2013:581093. PMCID: PMC3893739
2. Kuo W-HK, Chase HA. Exploiting the interactions between poly-histidine fusion tags and immobilized metal ions. Biotechnol Lett. 2011 Jun 1;33(6):1075–1084.
3. Baer S, Nigro J, Madej MP, Nisbet RM, Suryadinata R, Coia G, Hong LPT, Adams TE, Williams CC, Nuttall SD. Comparison of alternative nucleophiles for Sortase A-mediated bioconjugation and application in neuronal cell labelling. Org Biomol Chem. 2014 May 7;12(17):2675–2685. PMID: 24643508
4. Woestenenk EA, Hammarström M, van den Berg S, Härd T, Berglund H. His tag effect on solubility of human proteins produced in Escherichia coli: a comparison between four expression vectors. J Struct Func Genom. 2004 Sep 1;5(3):217–229.
5. Impact of an N-terminal Polyhistidine Tag on Protein Thermal Stability | ACS Omega [Internet]. [cited 2021 Apr 19]. Available from: https://pubs.acs.org/doi/10.1021/acsomega.7b01598
6. Coyle BL, Baneyx F. A cleavable silica-binding affinity tag for rapid and inexpensive protein purification. Biotechnol Bioeng. 2014 Oct; 111(10):2019–2026. PMID: 24777569
7. J S-R, Bl C, A S, K A, F B. Affinity purification of Car9-tagged proteins on silica matrices: Optimization of a rapid and inexpensive protein purification technology. Protein Expr Purif. 2017 May 12;135:70–77. PMID: 28506644