Application Note

Simultaneous cell lysis and capture using the Amicon® Pro device expedites recombinant protein purification

Introduction

E. coli transformation with poly-histidine tagged constructs represents a common vehicle for protein production. Initial screening of small-scale cultures is routinely performed to identify clones producing the highest amounts of protein with the desired form and function. As the demand for greater throughput at this level has increased, so has the need for process simplification and greater reproducibility. To meet this need, we have developed a condensed workflow that can be performed in a single device.

The standard purification workflow consists of three phases; cell lysis, clarification, and affinity purification (Figure 1). Traditional mechanical lysis methods, involving freeze/thaw or sonication cycles, are tedious and harsh, leading to diminished protein integrity and prep-to-prep variability. Agarose resin-packed gravity and spin-columns provide vehicles for affinity purification. However, these methods are hampered by concerns over sample purity and tedious workflows, respectively.

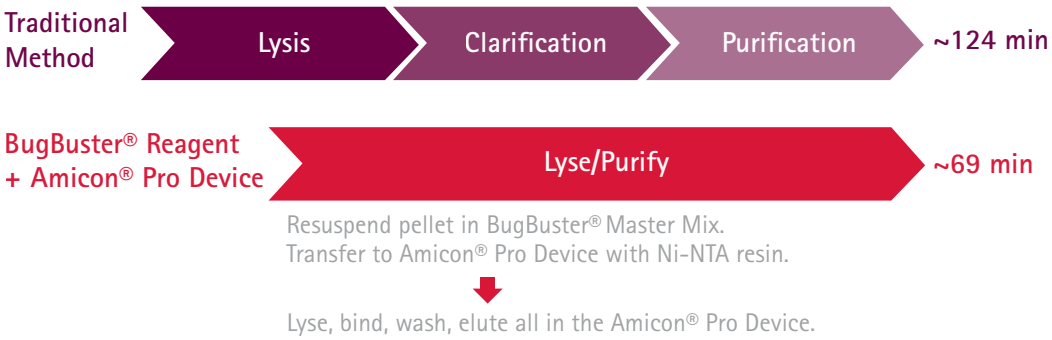

Figure 1.

Condensing the recombinant protein purification workflow. Outlined are workflow schematics for traditional purification involving mechanical lysis (sonication), and affinity purification. A condensed workflow combining chemical lysis (using BugBuster® Master Mix) and affinity capture step with purification all performed within the Amicon® Pro device.

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Here, we demonstrate a condensed workflow, combining the bacterial cell lysis and affinity capture steps (Figure 1). This is made possible by the gentle, detergent-based BugBuster® Master Mix lysis reagent[1] and the Amicon® Pro purification device, a centrifugal-based affinity resin purification system.

By enabling the extraction and purification procedures to be performed in a single device, the Amicon® Pro system eliminates the need for multiple sample transfers, thereby minimizing protein loss.

Methods

Protein expression

50 mL sterile LB-AMP media (1x Luria Broth + 100 µg/mL Ampicillin) was inoculated with one *E. coli* (strain BL21[DE3], Cat. No. 69450-4) colony expressing His-GAPDH (glyceraldehyde 3-phosphate dehydrogenase). The culture flask was incubated at 37 °C with 300 rpm shaking until A_{600} = 0.5 to 1.0. For induction, 12.5 mL of log-phase starter culture was added to 500 mL of Overnight Express™ Instant LB Medium (Cat. No. 71757-4) + 100 µg/mL AMP. Cultures were incubated at 37 °C with 300 rpm shaking for 16 h. Aliquots of the culture (various volumes) were dispensed into 15 or 50 mL tubes and centrifuged at 5000 rpm for 20 minutes. Wet cell pellet mass was determined and aliquots were frozen at -20 °C until lysis.

Preparation of lysates

Chemical-based lysis was performed using BugBuster® Master Mix (Cat. No. 71456) according to the user manual. BugBuster® Master Mix combines BugBuster® extraction reagent with enzymatic agents, Benzonase® nuclease and rLysozyme™ solution, in one formulation. Cell pellets were resuspended in lysis buffer (in general, 0.5 mL BugBuster® Master Mix/2.5 mL culture) and incubated at room temperature for 30 min with gentle agitation. Insoluble cell debris was cleared by centrifugation at 9500 rpm for 20 min. Supernatant was removed, aliquoted, and stored at -20 °C.

Purification of His-GAPDH from lysate using the Amicon® Pro device

His-GAPDH was purified from *E. coli* lysate using the Amicon® Pro Affinity Concentration Kit – Ni-NTA (Cat. No. ACR5000NT) according to the user manual[2-3]. Purification was done in the absence of the Amicon® Ultra 0.5 filter; only the bind, wash, and elution steps were performed. Briefly, 0.5 mL *E. coli* lysate containing His-GAPDH was incubated with 100 µL His•Bind® resin in an Amicon® Pro device for 60 min with gentle agitation. Unbound sample was cleared (1000 g for 1 min). 1.5 mL Wash Buffer was added to the resin and centrifuged (1000 g for 1 min). The resin was gently resuspended in 0.5 mL Elution Buffer, centrifuged at 1000 g for 1 min, and recovered from the collection tube.

His-GAPDH purification by combined cell lysis and capture in the Amicon® Pro device

A step-by-step protocol is provided:

- In the Amicon® Pro device, wash 200 µL His•Bind® resin slurry with 0.5 mL equilibrium buffer by centrifugation (1000 g for 1 min).
- Resuspend cell pellets (from culture volumes, 2.5-40 mL) in 0.5 (or 2) mL BugBuster® Master Mix and transfer to the device containing His•Bind® resin.
- Mix samples by gentle agitation for 1 hour.
- Clear unbound protein by centrifugation (1000 g for 1 min).
- Wash resin with 1.5 mL wash buffer by centrifugation (1000 g for 1 min). Empty the collection tube or replace with a clean 50 mL conical tube.
- Gently resuspend the resin in 0.5 mL elution buffer, centrifuge at 1000 g for 1 minute.
- Recover eluted fraction from the collection tube.

Electrophoresis and staining

Samples in 1X SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) loading buffer were denatured at 70 °C for 10 min and loaded onto 4-12% gradient NuPAGE® Bis-Tris (MES) Gels (Life Technologies) with 5 µL of protein molecular weight standards. Gels were run at 200 V for 40 min, stained/destained with SimplyBlue™ Safe Stain (Life Technologies) using the microwave method and imaged. Gel band intensity was quantified using MultiGauge v2 software (FujiFilm).

Results

Previous work using BugBuster® Master Mix for simultaneous cell lysis and affinity capture by PureProteome™ Nickel Magnetic beads showed good success for protein recovery relative to the standard multi-step method (lyse then capture)[4]. Building on these findings, we assessed the potential for combining the same steps in an agarose-based purification scheme using the Amicon® Pro device. Equivalent pellet aliquots (80 mg ≈10 mL) from a bulk culture (expressing His-GAPDH) were resuspended in BugBuster® Master Mix. Samples were processed following two workflows: (1) **Lyse then Capture** - standard 30 minute lysis in a microcentrifuge tube, 20 minute centrifugal clarification, and 1 hour affinity capture within the Amicon® Pro device and (2) **Lyse and Capture** – combined 1 hour lysis and capture in the Amicon® Pro device. All downstream steps (flowthrough clearance, wash, and elution) were performed within the Amicon® Pro device and were identical for the two protocols.

Overall, the combined Lyse and Capture method streamlined the workflow (elimination of the separate lysis and debris clarification steps) and reduced processing time by 44% (69 vs. 124 minutes). Critical to this process was the inclusion of Benzonase® Nuclease in the lysis reaction; if standard BugBuster® extraction reagent alone was used, the 80 mg pellet samples could not be processed because the devices clogged due to

high viscosity caused by release of bacterial nucleic acids. In fact, an Amicon® Pro device was able to effectively process up to 320 mg wet pellet mass (approximately 50 mL culture) following lysis in 0.5 mL BugBuster® Master Mix (data not shown). Note that this capability of manipulating culture volumes up to 50 mL without clogging is based on an observation made using a single *E. coli* strain; if different strains are to be used, prior optimization of reaction parameters (reaction volume, resin input, reaction time and method) is recommended. More significantly, process improvements had no adverse effects on final protein yield or purity. A representative gel is presented in Figure 2. Looking specifically at the flowthrough fractions, there is little difference between the two methods when comparing the degree of protein liberation (Lane 1s and Figure 3). Both the wash and elution fractions showed similar protein content (Lane 2s and 3s, respectively) suggesting that the presence of cell debris in the upper exchange chamber did not contribute to any greater amount of nonspecific binding.

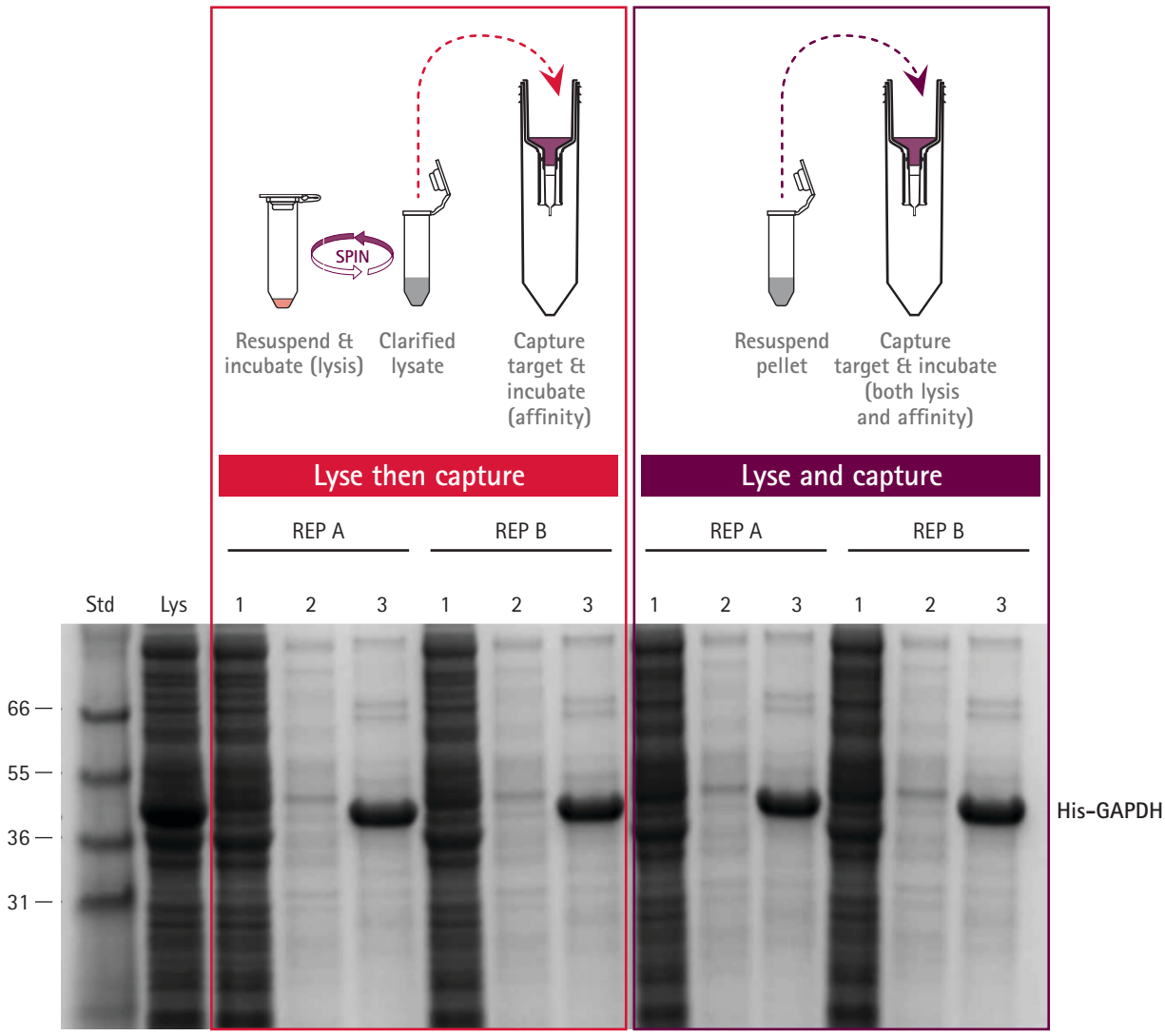

Figure 2.

Fraction profiles following purification via standard Lyse then Capture and one-step Lyse and Capture techniques. For Lyse then Capture, a cell pellet was resuspended in 0.5 mL BugBuster® Master Mix, lysed by gentle agitation for 30 minutes, and clarified by centrifugation. The resulting lysate was subject to purification using His•Bind® resin in the Amicon® Pro device. For Lyse and Capture, a replicate pellet was resuspended in 0.5 mL BugBuster® Master Mix, transferred to an Amicon® Pro device, mixed with resin for 1 hour, and purified using the standard protocol. The resulting fractions were run on SDS-PAGE and stained to assess relative yield and purity. The lanes correspond to: Std - molecular weight standard, Lys - lysate pre-passage, 1 - flowthrough, 2 – wash fraction, and 3 – elution fraction. Two replicate preps are shown for each method.

On average, the two methods resulted in roughly equivalent yield of His-GAPDH in final eluates (Figure 3). Interestingly, if the cell pellets were initially lysed in 2 mL (vs. 0.5 mL) of BugBuster® Master Mix, a higher level of His-GAPDH recovery (20%) was seen for the combined method alone. This finding suggests that increasing the reaction mix volume may enhance the affinity capture process potentially through either dilution of cell debris or a reduction in nonspecific binding. It should be noted that while this study was limited to the purification phase, the combined method does not preclude the use of Amicon® Ultra 0.5 filters during the elution phase.

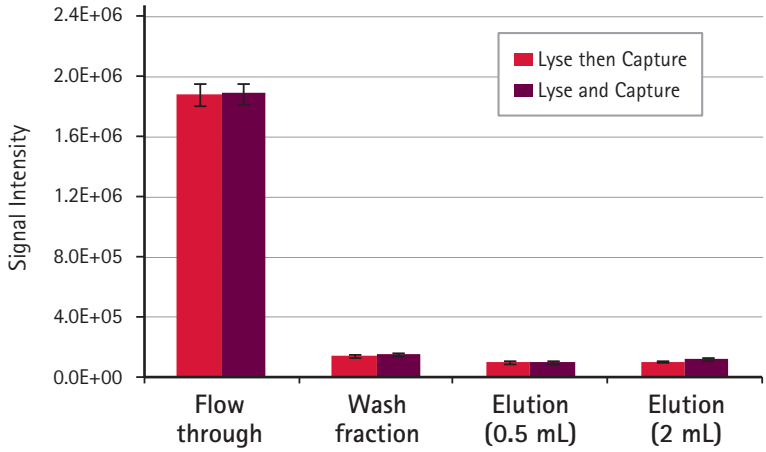

Figure 3.

Relative protein content in the three purification fractions (flowthrough, wash, and elution) was determined by densitometric quantitation of gel bands for the two methods outlined in Figure 2. For each fraction, the bars represent the average and standard deviation of four individual replicate purifications. For the two elution fractions, 0.5 and 2 mL represent the volume of BugBuster® Master Mix used in the reaction.

Summary

Speed, ease of use, and assay reproducibility are key parameters to be considered when expanding the throughput capacity of a given application. For the purification of recombinant proteins, initial screening is performed to identify the clone with optimal properties; for this process to be conclusive, all proteins in the screen must be purified under identical conditions, requiring an increase in protein purification throughput.

In this report, the protein purification workflow has been streamlined by simultaneously performing chemical lysis, using BugBuster® Master Mix, and agarose-based affinity purification. Following resuspension of bacterial cell pellets, all steps were performed within the Amicon® Pro device following standard procedures for protein purification. The result is a reduction in the number of process steps (including elimination of the clarification spin) as well as overall process time (69 minutes) without sacrificing yield or sample purity. Key to this modified protocol is the reduction in sample viscosity brought about by the use of Benzonase® nuclease. Further, confinement of the sample to a single device during the entire transformation of cell pellet to purified protein eliminates the risk of sample loss and minimizes sample-to-sample variation. Lastly, final sample formulation (buffer exchange and concentration) can be easily achieved in a single 15 minute spin through the attachment of an Amicon® Ultra 0.5 mL filter to the base of the Amicon® Pro device.[2]

References:

1. Simplifying the Bacterial Recombinant Protein Workflow while Maximizing Recovery. 2012. EMD Millipore Application Note, Literature Number AN3302EN00.
2. The Amicon® Pro purification system: condensing the protein purification workflow into one centrifugal device. 2013. EMD Millipore Application Note, Literature Number AN5588EN00.
3. User guide for the Amicon® Pro Affinity Concentration Kit – Ni-NTA. EMD Millipore, Literature Number BWENT Rev A., 2013.
4. Automated purification of proteins from non-clarified *E. coli* lysate using BugBuster® Master Mix and PureProteome™ Nickel Magnetic Beads. 2013. EMD Millipore Application Note, Literature Number AN9377EN00.

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Description	Catalogue No.
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