Application Note

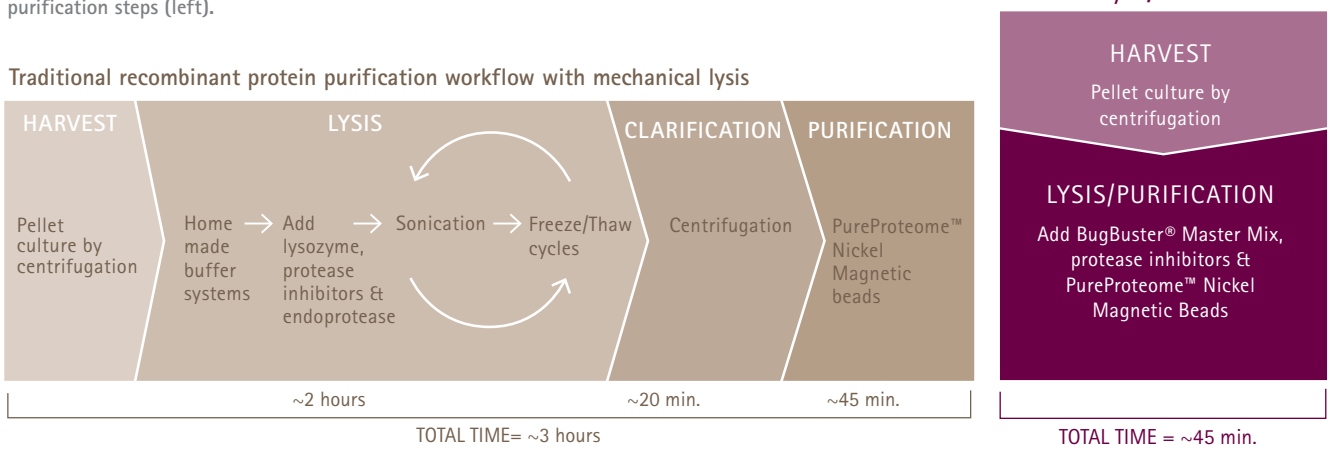
New, combined lysis and purification reaction simplifies recombinant protein purification with magnetic beads

Introduction

Traditionally, protein purification from *E. coli* consists of four distinct phases: harvest, bacterial cell lysis, lysate clarification and protein purification. Bacterial lysis typically requires several time-consuming, hands-on steps, such as freeze/thaw cycles and sonication. These harsh lysis techniques may negatively impact protein quality and contribute to sample-to-sample variability. To maintain protein activity and integrity, detergent-based lysis buffers are routinely used to avoid mechanical protein extraction methods. Regardless of the lysis method used, centrifugation is traditionally required to pellet unwanted cell debris and permit recovery of the clarified lysate. The final step, purification, is frequently performed using affinity media specific for expressed epitope tags. Agarose-based media have typically been used, either as a slurry in microcentrifuge tubes or packed into gravity-driven or spin columns. While easier to manipulate, columns are greatly affected by lysate consistency and carryover of cell debris, which can lead to clogging of the column frits.

In this application note, we demonstrate a new protocol for condensing the traditional recombinant protein purification workflow by combining enzymatic lysis and purification steps (Figure 1). This results in significantly less hands-on time and a greater than two-hour time savings over the traditional workflow. Using a magnetic bead approach for capture eliminates the need to clarify lysates by centrifugation. This adaptation facilitates automation of the developed protocol using the KingFisher® particle processor, resulting in increased sample throughput while further reducing actual hands-on processing time to less than 10 minutes. To further enable the condensed workflow, we used BugBuster® Master Mix reagent, which allows for non-mechanical extraction of soluble protein from bacterial cells. This extraction reagent combines detergent-based lysis with the enzymatic agents, Benzonase® nuclease and rLysozyme™ enzyme, in a ready-to-use formulation.

Figure 1. One-step lysis combined with purification (right) saves considerable time compared to traditional recombinant protein purification, which requires separate lysis, lysate clarification and purification steps (left).



EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

In recent years, magnetic beads have been accepted in applications where agarose beads have been typically used, reducing processing time and increasing sample throughput. Magnetic beads are generally used in batch mode, and are isolated on a magnet (either manually or using an automation platform) to allow for exchange of sample or wash buffer. PureProteome™ Nickel Magnetic Beads are optimized for the capture of histidine-tagged proteins. They generate recombinant proteins at high purity and can be used manually or on automated systems.

Materials and Methods

Histidine-tagged recombinant glyceraldehyde phosphate dehydrogenase (GAPDH) was purified with PureProteome™ Nickel Magnetic Beads, using a traditional purification workflow (mechanical lysis) and the new condensed protocol (combined enzymatic lysis and purification). We also compared the results of manual processing with results obtained using the KingFisher® automated particle processor.

Traditional Manual Protein Purification Using Mechanical Lysis

1 mL of *E. coli* culture was pelleted into 1.5 mL microcentrifuge tubes (6 replicates) and the supernatant was discarded. 100 µL of lysis/wash buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 8) containing 0.1 mg/mL lysozyme was added to each pellet. The pellet was resuspended and incubated at room temperature for 30 minutes with end–over-end mixing, followed by sonication (3 cycles of 10 seconds on, 30 seconds off, at medium power while on ice) using a microtip. The lysate was frozen, followed by quickly thawing at 37 °C. The sonication/freeze-thaw cycle was repeated once more. To reduce the viscosity, Benzonase® endonuclease was added to the lysate and incubated for 10 minutes at room temperature. The lysate was clarified by centrifugation at 10,000 x g for 20 minutes.

The clarified lysate was added to PureProteome™ Nickel Magnetic Beads (corresponding to 50 µL of suspended slurry, Cat. No. LSKMAGH10) that had been previously washed with 500 µL of wash buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 8). The PureProteome™ Magnetic Stand (Cat. No. LSKMAGS08) was used for bead capture steps to allow for exchange of solutions from each sample. The beads were incubated with *E. coli* lysate for 30 minutes with end–over-end mixing at room temperature. After removal of the lysate, the beads were washed with 500 µL of lysis/wash buffer by vortexing for 10 seconds, capturing the beads on the magnet and removing the buffer with a pipette. The wash step was repeated two more times prior to eluting the captured histidine-tagged GAPDH with the addition of 100 µL of elution buffer (50 mM sodium phosphate, 300 mM sodium chloride, 300 mM imidazole, pH 8). The beads were mixed at room temperature for 1-2 minutes. An additional elution was performed in a similar manner with 50 µL to achieve maximum yield. Both elution fractions were combined into the same microcentrifuge tube and saved for further analysis.

One-Step Protein Purification Using Combined Enzymatic Lysis and Purification

Condensed Protocol, Manual Processing

1 mL of *E. coli* culture was pelleted into 1.5 mL microcentrifuge tubes (6 replicates) and the supernatant was discarded. 310 µL of suspended PureProteome™ Nickel Magnetic Bead slurry (Cat. No. LSKMAGH10) was added to a separate 1.5 mL microcentrifuge tube. Using the PureProteome™ Magnetic Stand (Cat. No. LSKMAGS08), the preservative was removed and the beads were washed with 750 µL of wash buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 8) by vigorously vortexing for 10 seconds. The beads were collected on the magnet, and the buffer was removed with a pipette. The beads (62 µL settled beads) were resuspended in 588 µL BugBuster® Master Mix (Cat. No. 71456-4) and 105 µL of the resulting suspension was added to each of the six *E. coli* pellets. Each tube was briefly vortexed then incubated at room temperature with end over end mixing for 30 minutes. The unbound fractions were discarded and as described above and beads were washed 3 times with 500 µL of wash buffer using the PureProteome™ Magnetic Stand to capture beads. Elution was performed as previously described.

Condensed Protocol, Automated Processing

Similar to the condensed manual protocol, 1 mL of *E. coli* culture was pelleted into Row D of a KingFisher® Microtiter Deepwell 96 plate (12 replicates) and the supernatant was discarded. A protocol was set up on KingFisher® Duo System (Thermo Fisher) according to the plate layout and conditions (mixing and collecting parameters for PureProteome™ magnetic beads) outlined in Table 1. Reagents and samples were pipetted into the KingFisher® Duo plates (Microtiter Deepwell 96 plate and elution strips) and 50 µL of suspended PureProteome™ Nickel Magnetic Bead slurry was brought up to a total volume of 200 µL with wash buffer to obtain sufficient volume for use with KingFisher® Duo System. Wash buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole,

pH 8) was used for both equilibration and wash steps and the elution buffer (50 mM sodium phosphate, 300 mM sodium chloride, 300 mM imidazole, pH 8) was pipetted into the elution strips. The protocol was executed and the plates were loaded into the KingFisher® Duo System. The PureProteome™ Nickel beads were equilibrated in wash buffer to remove preservatives. The beads were then collected and placed in row D containing the *E. coli* pellet and BugBuster® Master Mix. Cell lysis and His-tagged protein capture was performed for 30 minutes. Following the lysis/binding step, the beads were washed and the recombinant protein was eluted. After the run was completed, the plates were removed and eluted sample fractions were collected and combined for further analysis.

Plate/Row or Elution Strip	Row Name	Content	Volume	Mixing Time/Speed	Collecting Count/Time
1-A	Beads	Beads + Wash Buffer	50 µL+150 µL	1 min/Medium	4/10 sec
1-B	Tip	12-Tip Comb	-	-	-
1-C	Equilibration	Wash Buffer	500 µL	1 min/Medium	4/10 sec
1-D	Lysis/Bind	*E. coli* Pellet + BugBuster® reagent	*E. coli* Pellet + 100 µL	30 min/Medium	4/10 sec
1-E	Wash 1	Wash Buffer	500 µL	1 min/Medium	4/10 sec
1-F	Wash 2	Wash Buffer	500 µL	1 min/Medium	4/10 sec
1-G	Wash 3	Wash Buffer	500 µL	1 min/Medium	4/10 sec
Elution Strip 1	Elution 1	Elution Buffer	100 µL	1 min/Medium	4/10 sec
Elution Strip 2	Elution 2	Elution Buffer	50 µL	1 min/Medium	4/10 sec

Table 1.
Pipetting, mixing and collecting instructions for His-tagged protein purification using the KingFisher® Duo System.

Determination of Total Protein Content In Lysates

Additional lysates were prepared using the traditional mechanical lysis (n= 6) approach as well as using the BugBuster® Master Mix (n=6) protocol as described earlier. In this case no magnetic beads were added during the lysis step. In both cases, the lysate was clarified by centrifugation to allow for analysis. The total protein concentration for each lysate was determined with the Direct Detect® IR-based spectrometer (Cat. No. DDHW00010-WW).

Determination of Protein Yield in Eluted Fractions

A quantitative Bradford assay was performed on the eluted fractions to assess the efficiency of lysis and protein purification.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Purified samples (10 µL of the eluted fractions) were reduced and denatured at 70 ° C for 10 min and loaded onto 1 mm thick 4–12% gradient NuPAGE® Bis-Tris gels (Life Technologies). Gels were run at 200 V for 35 min. Following electrophoresis, the gels were stained with Coomassie®-blue to visualize protein bands.

Results

Total Protein in Lysates

Lysates prepared using traditional mechanical lysis as well as with BugBuster® Master Mix were analyzed by the Direct Detect® spectrometer for total protein yield to serve as a control for the proposed workflow improvements. The results demonstrate that BugBuster® Master Mix lysis reagent provided more total protein in less time and with greater consistency than using the traditional method (Table 2).

Lysis Method	No. of Samples	Average Total Protein (mg/mL)	% CV	Lysis Time (min)
Traditional Mechanical	6	3.54	15.55	~120
BugBuster® Master Mix	6	4.09	2.05	~30

Table 2.
Total protein concentration measurements of *E. coli* lysates using the Direct Detect® spectrometer, comparing traditional mechanical lysis with enzymatic lysis using BugBuster® Master Mix.

Purified Protein Yield and Reproducibility

The eluted fractions from all workflows were tested for protein yield using a Bradford assay. On average, all three workflows demonstrated roughly equivalent yields of purified protein. However, the traditional method exhibited far greater inter-sample variability than did either condensed protocol. Not surprisingly, workflow automation using the Kingfisher® Duo particle processor offered the highest degree of reproducibility in sample preparation.

Workflow	Mode	No. of Samples	Average Amount of Purified Protein (µg)	% CV
Traditional	Manual	6	44.96	16.39
Condensed	Manual	6	50.49	7.74
Condensed	Automated	12	48.37	4.93

Table 3.
Yield of His-tagged GAPDH using the traditional and condensed *E. coli* lysis and purification protocol as determined by Bradford assay.

SDS-PAGE

Visual inspection of the Coomassie®-stained SDS-Page gel, demonstrated that all workflows provided similar sample purity. As previously noted, greater sample-to-sample variability was observed for the traditional recombinant protein purification workflow with mechanical lysis.

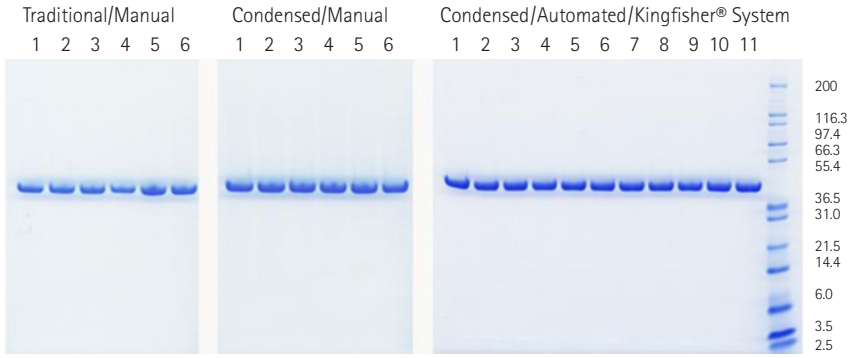


Figure 2. SDS-PAGE analysis of His-tagged GAPDH purified using the traditional and condensed recombinant protein purification workflows. The condensed protocol was performed manually as well as on the KingFisher® Duo System. (Molecular weight standards are included in the rightmost lane).

Discussion

For extraction of recombinant protein from *E. coli*, traditional mechanical lysis protocols are long (≥ 3 hours) and have many hands-on steps. We have shown that mechanical lysis and manual processing resulted in highly variable protein yield. Most likely, sample-to-sample variations in the sonication step contributed significantly to the lack of reproducibility. By combining gentle non-mechanical lysis (using BugBuster® Master Mix reagent) with PureProteome™ Nickel Magnetic Beads for His-tagged protein purification, the traditional recombinant protein purification workflow has been condensed.

Even when samples were manually processed, a one-step lysis and purification protocol reduced processing time by 75% (45 minutes vs. 3 hours) without sacrificing yield or purity. Due to reduced sample manipulation, the simplified protocol also provides greater consistency from prep to prep. Moreover, by eliminating the need for centrifugation (clarification step), the condensed workflow can be automated using systems such as the KingFisher® particle processors to further reduce sample variability and increase throughput while reducing actual hands-on time to less than 10 minutes.

Ordering Information

Description	Qty/Pk	Catalogue No.
PureProteome™ Protein A Magnetic Beads	2 x 1 mL	LSKMAGA02
	1 x 10 mL	LSKMAGA10
PureProteome™ Protein G Magnetic Beads	2 x 1 mL	LSKMAGG02
	1 x 10 mL	LSKMAGG10
PureProteome™ Protein A/G Mix Magnetic Beads	2 x 1 mL	LSKMAGAG02
	1 x 10 mL	LSKMAGAG10
PureProteome™ Kappa Ig-Binder Magnetic Beads	2 x 1 mL	LSKMAGKP02
PureProteome™ Lambda Ig-Binder Magnetic Beads	2 x 1 mL	LSKMAGLM02
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08
BugBuster® Master Mix	100 mL	71456-3
	500 mL	71456-4
Direct Detect® Spectrometer	1	DDHW00010-WW

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