Application Note

Automated purification of proteins

from non-clarified *E.coli* lysate using BugBuster® Master Mix and PureProteome™ Nickel Magnetic Beads

Introduction

Purification of recombinant proteins expressed in *E.coli* requires many time-consuming steps. To liberate the protein of interest, traditional bacterial lysis relies on the addition of lysozyme and a combination of sonication and repeated freeze/thaw cycles to break the bacterial cell wall. Disruption of the cell is accompanied by an increase in the viscosity of the suspension, due to the release of DNA. An endonuclease is added to digest the DNA, thus reducing the viscosity of the lysate. Finally, to render the lysate compatible with traditional purification methods, insoluble cell debris must be removed by centrifugation.

In this application note, we use magnetic affinity beads to collapse the traditional recombinant protein purification workflow by integrating lysis with DNA digestion and by eliminating the lysate clarification step, requiring as little as 15 minutes of hands-on time for the entire process (Figure 1).

In recent years, magnetic beads have been accepted in applications where agarose beads have been typically used, reducing processing time, enabling automation and increasing sample throughput. In contrast to agarose beads, which require time-consuming centrifugation steps or lengthy separations on gravity columns, magnetic beads are generally used in batch mode, and are isolated on a magnet (either manually or using an automation platform) to allow for efficient removal of sample, wash buffers or eluate. Since magnetic beads do not require centrifugation, cell debris in nonclarified lysate does not affect bead performance.

Figure 1. One-step lysate preparation without clarification (Panel B) saves considerable time compared to traditional recombinant protein purification, which requires manual lysis and lysate clarification (Panel A).

We demonstrate the automated purification of histidine-tagged recombinant glyceraldehyde phosphate dehydrogenase (GAPDH) from non-clarified *E. coli* lysate with PureProteome™ Nickel Magnetic Beads using the KingFisher® Duo particle processor. BugBuster® Master Mix was used for preparation of lysates to reduce the typical lysate preparation time. This master mix combines BugBuster® Protein Extraction Reagent with Benzonase® Nuclease and rLysozyme™ reagents, providing a one-step solution for gentle disruption of the *E.coli* cell wall as well as reduction of lysate viscosity.

Materials and Methods

Bacterial Lysate Preparation

Two 100 mL aliquots of an *E. coli* culture (expressing recombinant 6x histidine-tagged GAPDH) were centrifuged at 10,000 x g for 20 minutes. Immediately after removal from the centrifuge, the supernatant was discarded.

One *E. coli* cell pellet (A) was lysed by resuspending in 10 mL of a traditionally recommended lysis buffer, consisting of 50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole pH 8. The suspension was supplemented with 0.1 mg/mL lysozyme and 10 µL of Protease Inhibitor Cocktail Set III (without EDTA) (Cat. No. 539134). Lysis was allowed to proceed on ice for 30 minutes. The cell suspension was then sonicated on ice and frozen at -80° C. This cycle was repeated until the lysate became translucent and viscous. Benzonase® nuclease was added to reduce the viscosity. The sample was split into two 5 mL aliquots, A1 and A2. Aliquot A1 was centrifuged at 10,000 x g for 20 minutes at 4° C to pellet the cell debris. The clarified lysate was removed and stored in a clean tube. No further processing was performed on aliquot A2 (non-clarified lysate). Lysates were stored frozen (-20° C) prior to use.

The second bacterial cell pellet (B) was lysed by resuspending in 10 mL BugBuster® Master Mix (Cat. No. 71456-4). The suspension was supplemented with 10 µL of Protease Inhibitor Cocktail Set III (without EDTA) (Cat. No. 539134). The cell suspension was incubated at room temperature using an end-over-end mixer for 20 minutes. The lysate was split into two 5 mL aliquots, B1 and B2. To prepare clarified lysate, aliquot B1 was centrifuged at 10,000 x g for 20 minutes at 4° C to remove insoluble cell debris. No further processing was performed on lysate aliquot B2. Both BugBuster® lysates were stored frozen (20° C) prior to use.

Table 1. Summary of the four different lysate preparations evaluated in this study.

Traditional lysis buffer	
A1 Clarified lysate	A2 Non-clarified lysate
BugBuster® Master Mix reagent	
B1 Clarified lysate	B2 Non-clarified lysate

Standard Manual Protocol for Recombinant GAPDH Purification

50 µL of suspended PureProteome™ Nickel Bead slurry (Cat. No. LSKMAGH10) was added into each of 24 - microcentrifuge tubes (1.5 mL). Using the PureProteome™ Magnetic Stand (Cat. No. LSKMAGS08), the beads were washed with 500 µL of wash buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 8) by vigorously vortexing for 10 seconds, collecting the beads on the magnet and removing the buffer with a pipette. 100 µL of samples A1, A2, B1 and B2 (6 replicates each) were added to the beads, and the mixture was incubated at room temperature for 30 minutes with continuous end–over-end mixing. The unbound fractions were discarded, and, as described above, the beads were washed 3 times with 500 µL of wash buffer using the PureProteome™ Magnetic Stand to capture beads. Elution was performed by adding 100 µL of elution buffer (50 mM sodium phosphate, 300 mM sodium chloride, 300 mM imidazole, pH 8) to the beads and mixing 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.

KingFisher® Duo Protocol for recombinant GAPDH purification

All the reagents and samples were pipetted into the KingFisher® Duo plates (Microtiter Deepwell 96 plate and elution strips) and a protocol was set up on KingFisher® Duo System (Thermo Fisher) according to the plate layout and optimized conditions (mixing and collecting parameters for PureProteome™ Magnetic Beads) outlined in Table 2. 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. 100 µL of clarified lysate with and without BugBuster® reagent (A1 and B1, 6 replicates each) were added to row D of the Microtiter Deepwell 96 plate. Wash buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 8) was used for both equilibration and washes 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 beads were equilibrated in wash buffer and mixed with 100 µL of lysate for 30 minutes. Following the 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. The same experiment was repeated with the non-clarified lysate with and without BugBuster® reagent (A2 and B2).

Plate/Row or Elution Strip	Row Name	Content	Volume	Mixing	Collecting
				Time/Speed	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	Bind	Non-clarified Lysate or BugBuster® Lysate	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 2. Pipetting, mixing and collecting instructions for the his-tagged protein purification using the KingFisher® Duo System.

Protein yield determination using Bradford Assay

10 µL of bovine serum albumin (BSA) standards and samples were pipetted into a microplate. 300 µL of Coomassie® Blue reagent was added to the microplate and the reactions were briefly mixed on a plate shaker. The absorbance of the samples at 595 nm was measured using a plate reader, and the concentration was determined using the BSA standard curve.

Electrophoresis and Coomassie® Staining

The eluted fractions were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to determine specificity of the elution and reproducibility. Purified samples (10 µL of the eluted fractions) and lysate (5 µL of starting material) 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). Proteins were separated at 200 V for 35 min. After electrophoresis, gels were removed from the cassette, briefly rinsed and washed 3 times with Milli-Q® water and stained with SimplyBlue™ SafeStain using the microwave protocol. Stained gels were then washed in Milli-Q® water for 10 minutes and imaged.

Results

We compared lysate preparation methods using traditional His-tagged protein purification lysis buffer and BugBuster® Master Mix. While traditional purification methods require lysate clarification, we explored the effect of non-clarified lysate on bead performance. Also, samples processed using standard manual protocol were compared to samples prepared via automated purification with the KingFisher® Duo particle processor.

Quantitation of total protein yield from the lysates via Bradford assay revealed that, for both clarified and non-clarified lysates and in both manual and automated processing protocols, more protein was liberated from cells with BugBuster® Master Mix than with traditional lysis buffer as shown in Figure 2. GAPDH purified using the KingFisher® automated processor contained comparable protein yields to manually generated lysates, and similar yields were obtained when the lysate clarification step was omitted.

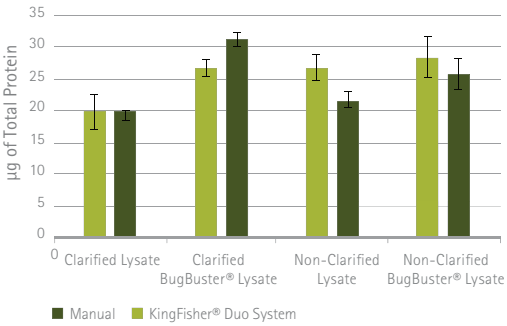

Figure 2.

BugBuster® Master Mix generates higher yields of total protein upon purification. Automated processing generated yields comparable to manual processing, and omitting the clarification step provided similar yields.

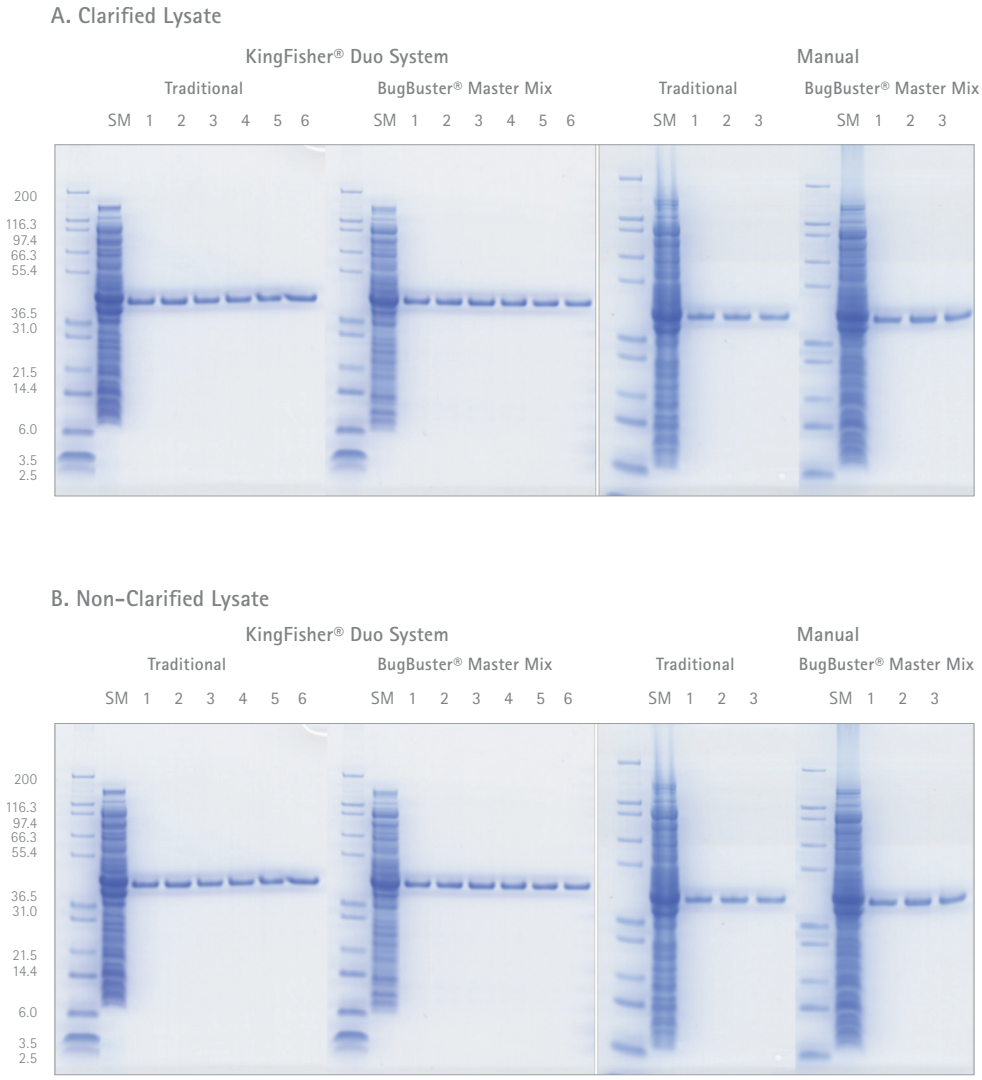

Figure 3.

Comparison of yield and purity of His-tagged protein from four different lysate preparations, clarified and non-clarified, traditional lysis buffer and BugBuster® Master Mix using automated or manual processing. Each gel shows (from left to right) molecular weight standard, starting material and replicate eluted fractions. Panel A shows the clarified lysate from traditional or BugBuster® Master Mix lysis, performed manually and using KingFisher® Duo System. Panel B shows non-clarified lysates purified in the same manner.

Visual inspection of the stained gels (Figure 3) indicated that, for both clarified and non-clarified lysates and in both manual and automated processing protocols, more His-tagged protein was purified when cells were lysed with BugBuster® Master Mix than when cells were treated with traditional lysis buffer.

All replicates from a given lysate preparation method contained protein of comparable yield and purity, indicating that PureProteome™ Nickel Magnetic Beads can be used to generate reproducible batches of recombinant protein via both automated and manual processing and that clarification is not necessary.

Conclusion

The results demonstrate that the PureProteome™ Nickel Magnetic Beads can be used for purification of recombinant protein from non-clarified lysate. The use of BugBuster® Master Mix eliminates cumbersome sonication and freeze/thaw steps typically associated with bacterial cell lysis. The use of the extraction reagent reduces sample handling requirements while providing greater protein yield.

Furthermore, His-tagged protein purification with PureProteome™ Nickel Magnetic Beads was successfully automated using the KingFisher® Duo bead handling system; resulting yields of purified protein were comparable to those obtained manually.

PureProteome™ Magnetic Beads are very well suited for an automated purification process using the KingFisher® particle processor, without requiring a lysate clarification step. Furthermore, the process is reproducible, and the results are comparable to that of a standard protocol using the PureProteome™ Magnetic Stand. PureProteome™ Magnetic Beads can be implemented on any automated system for low, medium or high-throughput sample preparation. We have successfully demonstrated a collapse in the traditional recombinant protein purification workflow.

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™ Nickel Magnetic Beads	2 x 1 mL	LSKMAGH02
	1 x 10 mL	LSKMAGH10
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08
BugBuster® Master Mix	100 mL	71456-3
	500 mL	71456-4

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Italy: 848 845 645
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