Novel Condensed Workflow Enabling High Throughput Recombinant Protein Purification from *E. coli*

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Abstract

Many scientists require purified proteins for their research applications. To meet this need, a multitude of recombinant expression techniques have been developed. While many expression systems are available, *E. coli* represents the most optimized and widely used vehicle for the expression of 6x Histidine-tagged recombinant proteins. Traditionally, the recombinant protein purification workflow consists of five distinct phases: Harvest, Lysis, Clarification, Purification and Analysis. Lysis typically requires multiple time-consuming, hands-on steps, such as freeze/thaw cycling and sonication, which may be harsh,may negatively impact protein quality and may contribute to sample variability. Clarification, which is primarily needed to reduce clogging in the downstream purification step, is typically performed in a centrifuge, making automation challenging. Purification is typically performed using columns packed with agarose beads with specificity for the tag on the recombinant protein. While columns provide an easy approach for manipulation of the affinity media, they are greatly affected by lysate consistency and carry-over of cell debris, which can lead to clogging and poor protein purification and recovery. To guarantee maximum recovery of protein activity and integrity, we have developed a protocol leveraging gentle, enzymatic cell lysis and purification with magnetic beads to condense the traditional His-tag protein purification workflow. This significantly improved workflow combines Lysis and Purification and eliminates the need for Clarification, resulting in a greater than two-hour time savings over the traditional workflow. In addition, this condensed workflow facilitates automation, resulting in increased sample throughput while further reducing actual hands-on processing time.

Introduction

The traditional workflow for 6x Histidine-tagged recombinant proteins from *E. coli* requires harsh mechanical lysis techniques which are labor intensive and may negatively impact protein quality and contribute to sample-to-sample variability. Here we demonstrate a One-Step protocol which condenses the traditional recombinant protein purification workflow by combining enzymatic lysis using BugBuster® Master Mix and affinity purification with PureProteome™ Nickel Magnetic beads into a single step. By using magnetic beads, the need for lysate clarification is eliminated. The One-Step protocol was further optimized for automated processing using a KingFisher® particle processor to reduce hands-on time and increase sample throughput. High throughput applications such as expression clone screening would greatly benefit from this streamlined workflow.

Materials & Methods

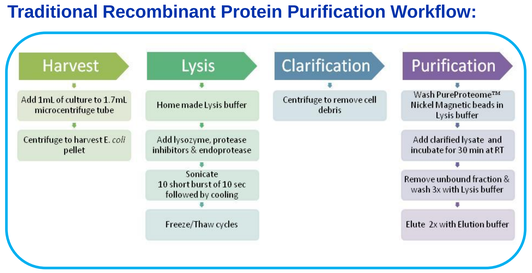
Recombinant protein expression using *E. coli*: Histidine -tagged recombinant glyceraldehyde 3-phosphate dehydrogenase (GAPDH) w expressed in *E. coli.* For harvesting, 1 mL aliquots of culture were transferred into 1.7 mL microcentrifuge tubes or a specified row of wells of a 96-well KingFisher® deepwell plate. Cells were pelleted by centrifugation and the supernernatant was discarded. Pellets were stored at -80 ºC until needed.

Reagents:

Reagent	Traditional Protocol (Manual)	One-Step Protocol (Manual)	One-Step Protocol (Automated)
Lysis buffer	50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole pH 8.0	BugBuster® MasterMix	BugBuster® MasterMix
Wash buffer	50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole pH 8.0		
Elution buffer	50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole pH 8.0		
Magnetic beads	50 µL of PureProteome™ Nickel Magnetic Bead slurry		

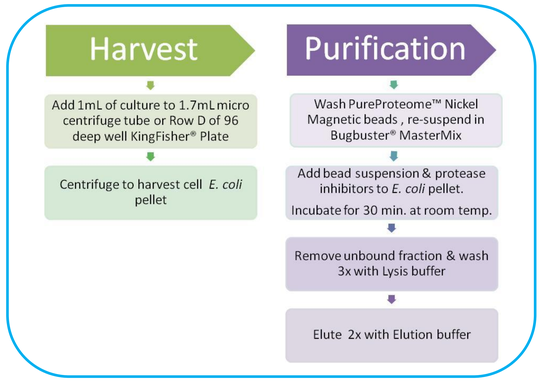
Manual Magnetic Bead Manipulation: The PureProteome™ magnetic stand was used for all magnetic bead manipulation. All magnetic bead wash steps were performed with mixing for 1 min. Beads were incubated with the *E. coli* lysate for capture of the recombinant GAPDH. After washing, the purified protein was eluted twice for 1 minute each.

Traditional Recombinant Protein Purification Workflow:



The traditional workflow consists of many time-consuming steps that can introduce sample variability. Processing time can be as long as ~180 minutes.

One-Step Purification Workflow



The One-Step Purification workflow reduces the total processing time to as little as ***45 minutes*** while providing greater sample consistency.

Automation of One-Step Purification Workflow on Magnetic Particle Handler

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

The One-Step purification protocol was optimized utilizing a KingFisher® Duo particle handler. The system allows for 12 individual purifications in parallel. Procedural steps established in the manual protocol were translated to work within this automated format.The above plate map provides conditions and reagent placement for One-Step purification on KingFisher® Duo. Following this approach, the actual “hands-on time” can be reduced to as little as 10 minutes.

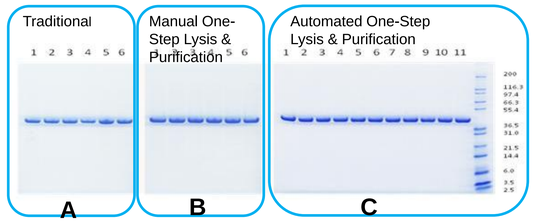
Results:

Protein Yield Based on *E.coli* Lysis Method

Lysis method	No. samples	Total protein liberated (mg/mL)	% CV	Lysis time (min)
Traditional / Mechanical	6	3.54	15.6	~120
BugBuster® MasterMix	6	4.09	2.05	~30

To compare the efficiency of the traditional mechanical lysis and detergent-based lysis with BugBuster® MasterMix reagent, the total protein concentration of *E. coli* lysates was measured using the Direct Detect® spectrometer. Both traditional as well as BugBuster® Master Mix lysates were clarified by centrifugation prior to measuring the protein content.

One-step Lysis & Purification Decreases Sample-toSample Variability



SDS-PAGE analysis of His-tagged GAPDH purified using the traditional (A) and One-Step recombinant protein purification workflows. The One-Step protocol was performed manually (B) as well as on the KingFisher® Duo System (C). Molecular weight standards are included in the rightmost lane. Greater sample-to-sample variability was observed for the traditional recombinant protein purification workflow with mechanical lysis.

One-step Lysis & Purification Increases Consistency of Protein Yield

Workflow	Mode	No. samples	Average µg protein purified	% CV
Traditional	Manual	6	44.96	16.39
One-Step	Manual	6	50.49	7.74
One-Step	Automated	12	48.37	4.93

Protein yield of His-tagged GAPDH using the traditional and One-Step E. coli lysis and purification protocol as determined by Bradford assay. All three workflows demonstrated roughly equivalent yields of purified protein. The traditional method exhibited far greater inter-sample variability than did either condensed protocol. Automated purification offered the highest degree of reproducibility in sample preparation.

Summary

- BugBuster® Master Mix provides a more efficient lysis method than traditional approaches resulting in improved protein liberation and less sample to sample variability.
- Combining the gentle, detergent-based lysis with BugBuster® MasterMix reagent with PureProteome™ Nickel Magnetic Beads for the purification of His-tagged proteins results in a significantly condensed workflow, without sacrificing yield or purity.
- Eliminating the need for clarification, the One-Step protocol enables complete automation on systems such as the KingFisher® to further reduce variability and increase throughput while reducing hands-on time to less than 10 minutes.

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