96 Wells, One Protein Purification System

HisLink™ 96 Protein Purification System: Fast Purification of Polyhistidine-Tagged Proteins

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Abstract

The HisLink™ 96 Protein Purification System allows efficient purification of polyhistidine-tagged proteins from 1ml of culture using a vacuum-based format. The system purifies polyhistidine-tagged proteins directly from bacterial, insect and mammalian cell culture without the need for prior processing of the culture or resin. Removing these prior processing steps results in a robust purification method that is easy to automate and adaptable to rapid screening processes.

The HisLink™ 96 Protein Purification System features a ready-to-use resin for purifying polyhistidine-tagged proteins directly from cell cultures.

Introduction

Due to the size, availability in many vector systems and ease of use for purification, the polyhistidine tag is a popular tool for purifying recombinant proteins. It is a robust tag that can be used under both native and denaturing conditions. Although the majority of the polyhistidine-tagged proteins are expressed in bacterial cultures, there is an increasing trend toward other expression systems, including insect or mammalian cell lines. The HisLink™ 96 Protein Purification System[a,b,c] is a multiwell protein purification system that can be used to purify proteins under either native or denaturing conditions and is equally efficient at purifying recombinant proteins from bacterial, insect and mammalian cell cultures. The high-throughput nature of the HisLink™ 96 Protein Purification System makes it an excellent choice for studying multiple recombinant proteins in parallel, allowing the quick determination of optimal growth or protein induction conditions.

HisLink™ 96 Protein Purification System

The HisLink™ 96 Protein Purification System purifies polyhistidine-tagged proteins directly from cultures grown in a 96-well, deep-well plate or 1ml samples. Figure 1 shows a schematic of protein purification using the HisLink™ 96 System. A mixture of FastBreak™ Cell Lysis Reagent[a,b,c] and DNase is added directly to the culture. An aliquot of settled HisLink™ resin is then added to the culture. The FastBreak™ Reagent efficiently lyses the cells, releasing proteins that can bind to the resin. After an incubation period the lysate and resin are transferred to a fritted Filtration Plate and captured. The addition of DNase reduces the viscosity of the lysate and decreases the potential for resin aggregration. The resin and protein complex are then washed, and the protein is eluted using a Vac-Man® 96 Vacuum Manifold (Figure 1).

Purifying Polyhistidine-Tagged Proteins from Bacterial Cells

Polyhistidine-tagged protein expression is commonly performed using bacterial cells. Variations in growth and protein induction conditions, including temperature and culture media, can affect cell culture density. Even within a single deep-well plate, internal variation can lead to a range of cell densities throughout the plate. To overcome potential purification problems caused by these variations, we designed the HisLink™ 96 Protein Purification System to purify protein over a broad range of cell densities up to 8.0 $O.D._{600}$ units per milliliter. Figure 2 shows polyhistidine-tagged Monster® Green Fluorescent Protein (hMGFP) purified from cultures ranging in density from 1.0–8.0 $O.D._{600}$ units per milliliter.

When comparing protein expression across a multiwell plate, it is beneficial to have consistent purification throughout the plate. We purified hMGFP from 1ml aliquots of a cell culture to show that there is low well-to-well variability when using the HisLink™ 96 System (Figure 3).

Proteins expressed in bacterial cells may end up in insoluble inclusion bodies. These insoluble proteins can be purified under denaturing conditions by adding 6M urea or guanidine to the cells. To test the ability of the HisLink™ 96 Protein Purification System to purify protein under denaturing conditions, we spiked polyhistidine-tagged firefly luciferase into a bacterial cell culture. The protein was purified following the standard protocol under denaturing conditions by adding 6M guanidine-HCl directly to the cell culture. To optimize purification under denaturing conditions, we added 500mM NaCl to the Wash Buffer. The yield of protein purified under these denaturing conditions was comparable to that purified under native conditions (Figure 4).

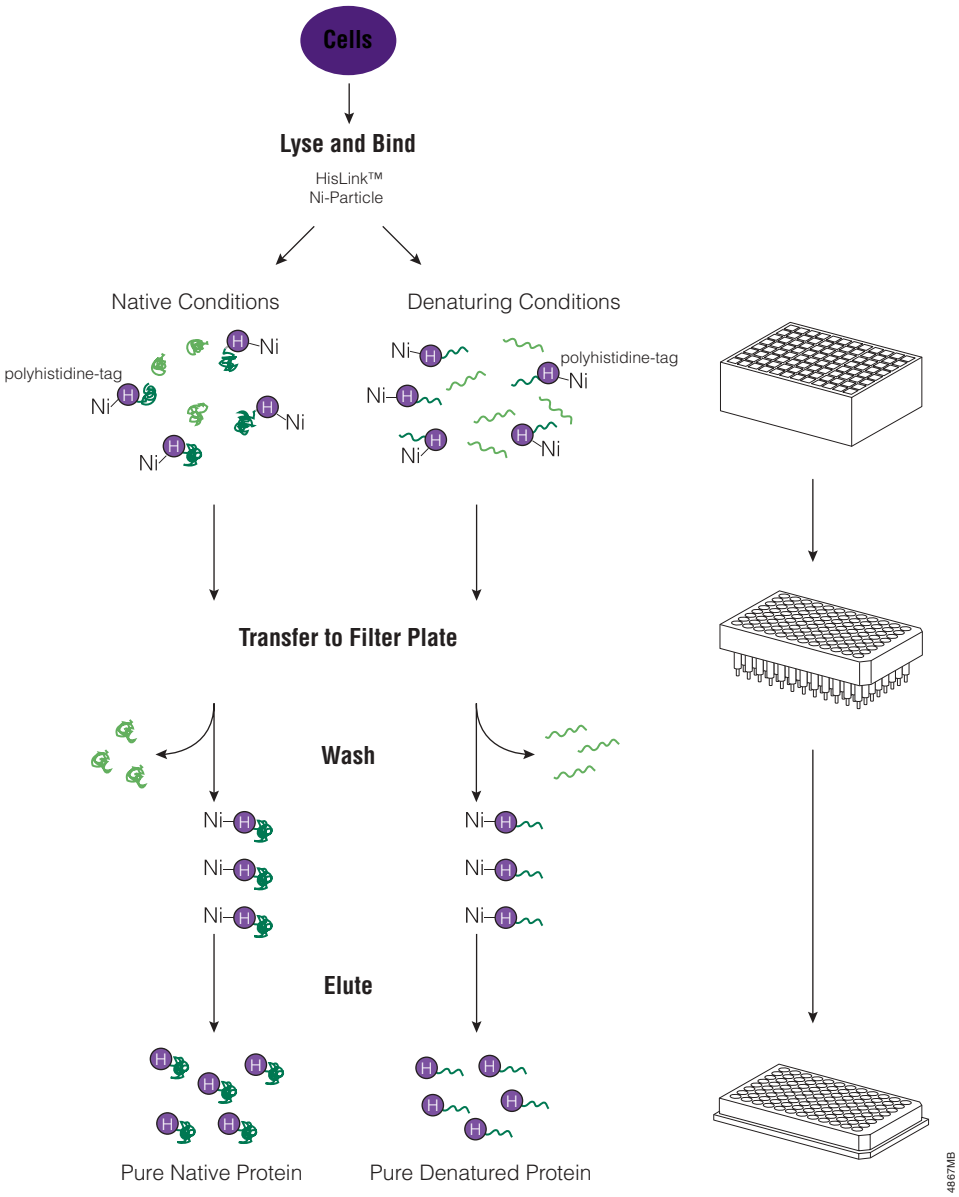

Figure 1. A schematic representation of the HisLink™ 96 Protein Purification Protocol.

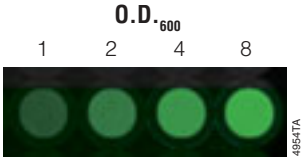

Figure 2. Monster Green® Fluorescent Protein purified from increasing densities of cell culture using the HisLink™ 96 Protein Purification System. Bacterial culture expressing polyhistidine-tagged hMGFP culture was diluted to 1, 2, 4 and 8 $O.D._{600}$ units per well using fresh Terrific Broth. Aliquots (1ml) were taken from each dilution and purified using the HisLink™ 96 Protein Purification System. Fluorescence from the purified protein was then visualized in the elution plate using a Typhoon™ 9410 workstation (excitation 488; emission filter 520BP40).

Figure 3. Consistent results with the HisLink™ 96 Protein Purification System. Aliquots (2.5 $O.D._{600}$ units per milliliter) of a bacterial culture expressing polyhistidine-tagged hMGFP were placed into 10 wells of a 96-well, deep-well plate. The samples were purified using the manual HisLink™ 96 method. Fluorescence from the eluted protein was scanned in the elution plate using a Typhoon™ 9410 workstation (CV <15%; excitation 488; emission filter 520BP40).

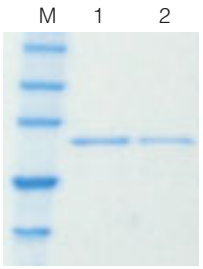

Figure 4. Polyhistidine-tagged firefly luciferase purified under native and denaturing conditions. Polyhistidine-tagged firefly luciferase was spiked into a bacterial cell culture, and 6M guanidine-HCl was added directly to some wells. The samples were then purified with the HisLink™ 96 System using a Biomek® 2000 instrument. To reduce the background commonly seen with denatured cell purification, 500mM NaCl was added to the wash buffer. Lane M, protein marker; lane 1, native lysing conditions (FastBreak™ Reagent); lane 2, denatured lysing conditions (FastBreak™ Reagent + 6M guanidine-HCl).

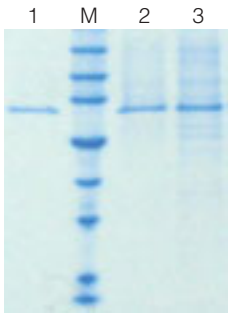

Figure 5. Polyhistidine-tagged firefly luciferase purified from Sf9 Insect cell lysates. Polyhistidine-tagged firefly luciferase was spiked into a Sf9 insect cell cultures with increasing cell numbers. The samples were then purified with the HisLink™ 96 System using a Biomek® 2000 instrument. To reduce the background, 500mM NaCl was added to the wash buffer. Lane M, protein marker; lane 1, growth media spiked with polyhistidine-tagged firefly luciferase; lane 2, 5×10^5 cells spiked with polyhistidine-tagged firefly luciferase; lane 3, 1.0×10^6 cells spiked with polyhistidine-tagged firefly luciferase.

Rapid Optimization of Protein Expression

An advantage of using a bacterial expression system is that both growth conditions and protein expression conditions can be optimized. Using the HisLink™ 96 Protein Purification System, several growth conditions can be tested on a single plate, and the polyhistidine-tagged proteins can be purified in parallel. This direct comparison of the conditions allows the optimal expression conditions to be determined quickly.

The HisLink™ 96 System can be used for the parallel expression and purification of multiple polyhistidine-tagged proteins on a single plate. This would allow the rapid screening of purified proteins for functional analysis.

Purifying Polyhistidine-Tagged Protein from Insect and Mammalian Cell Lines

Insect and mammalian cell expression systems can also be used to express polyhistidine-tagged proteins. The HisLink™ 96 Protein Purification System can be easily adapted to these expression systems. The FastBreak™ Cell Lysis Reagent has been shown to lyse bacterial, insect and mammalian cells (1,2). Thus the HisLink™ 96 System can be used to optimize expression conditions for insect and mammalian cell expression systems. This approach would potentially allow direct comparisons of bacterial-, insect- and mammalian-expressed proteins in a single purification experiment.

We demonstrated the utility of the HisLink™ 96 Protein Purification System to purify a polyhistidine-tagged protein from insect cell lysates. Sf9 insect cells (Invitrogen) were spiked with polyhistidine-tagged firefly luciferase and lysed using the FastBreak™ Cell Lysis Reagent. A 1ml aliquot of this lysate was placed into a well of a deep-well plate and purified using the HisLink™ 96 System. As a control, the polyhistidine-tagged protein was spiked into growth media and purified on the same plate. To optimize purification, we added 500mM NaCl to the Wash Buffer. The results show that polyhistidine-tagged luciferase was efficiently purified from the insect cell lysates with minimal nonspecific protein contamination as well as from the culture medium (Figure 5).

The HisLink™ 96 Protein Purification System can also be used to purify a polyhistidine-tagged protein from mammalian cell lysates. We spiked Chinese Hamster Ovary (CHO) cells with polyhistidine-tagged ubiquitin and lysed using the FastBreak™ Cell Lysis Reagent. Aliquots (1ml) of this lysate were placed into wells of a deep-well plate and purified using the HisLink™ 96 System. To optimize purification from the mammalian cell extract, we added 500mM NaCl to the Wash Buffer. Figure 6 shows that the polyhistidine-tagged ubiquitin was efficiently purified with minimal nonspecific protein contamination.

Automation Using the HisLink™ 96 Protein Purification System

The HisLink™ 96 Protein Purification System's ability to efficiently purify proteins directly from cell culture combined with the ease of use of the resin make the system simple to automate. To be adapted to a robotic platform, the system requires only a vacuum pump and wide-bore pipette tips. We have developed a method using the HisLink™ 96 System on a Beckman Coulter Biomek® 2000 instrument. This system can be programmed to be hands-off once the deck has been set up and the program is started. More information about the automated method is available online at: **www.promega.com/automethods/**

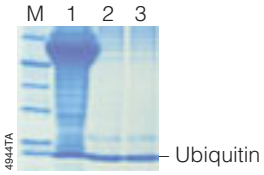

Figure 6. Polyhistidine-tagged ubiquitin purified from CHO cell lysates. Polyhistidine-tagged ubiquitin was spiked into 1.5×10^6 CHO cells. The samples were then purified with the HisLink™ 96 System using the manual protocol. To reduce the background, 500mM NaCl was added to the wash buffer. Lane M, protein marker; lane 1, crude lysate spiked with polyhistidine-tagged ubiquitin (pre-purification); lanes 2 and 3, purified polyhistidine-tagged ubiquitin.

Conclusion

The HisLink™ 96 Protein Purification System is a vacuum-based method for the purification of polyhistidine-tagged proteins directly from cell culture. Because the HisLink™ 96 System purifies polyhistidine-tagged proteins without extensive cell culture or resin preparation, it is easily automated. The use of DNase in the cell lysis step reduces lysate viscosity and allows protein purification from bacteria cell cultures with densities as great as 8.0 $O.D._{600}$ units without filter clogging. This flexible system purifies proteins from a variety of protein expression systems, including bacterial, insect and mammalian cell lines, under either native or denaturing conditions. The HisLink™ 96 System offers a quick and easy method that can be used for screening multiple cell growth and protein induction conditions, thus reducing the time required for optimizing protein expression.

References

1. Betz, N. (2004) *Promega Notes* **87**, 29–32.
2. Betz, N. (2004) *Cell Notes* **9**, 6–9.

Related Articles

1. Simpson, D. and Godat, B. (2004) *Promega Notes* **86**, 11–4.
2. Kobs, G. (2004) *Cell Notes* **9**, 2–4.

Protocols

- *HisLink™ 96 Protein Purification System Technical Bulletin* #TB342 Promega Corporation. (www.promega.com/tbs/tb342/tb342.html)
- *FastBreak™ Cell Lysis Reagent, 10X, Product Information* #9PIV857 Promega Corporation. (www.promega.com/tbs/9piv857/9piv857.html)

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Ordering Information

Product	Size	Cat.#
HisLink™ 96 Protein Purification System	1 × 96 reactions	V3680
	5 × 96 reactions	V3681
FastBreak™ Cell Lysis Reagent, 10X	15ml	V8571
	60ml	V8572
	100ml	V8573
Vac-Man® 96 Vacuum Manifold	1 each	A2291

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