HisLink™ Spin Protein Purification System: Maximum Versatility in a Small Package

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Abstract

The HisLink™ Spin Protein Purification System is capable of purifying 1mg of polyhistidine- or HQ-tagged proteins using a single spin column with either a vacuum manifold or a tabletop centrifuge. This system purifies protein directly from bacterial cell culture medium, making it a quick and easy purification tool.

The HisLink™ Spin Protein Purification System purifies polyhistidine- or HQ-tagged proteins from bacterial cell culture using either a centrifuge or a vacuum manifold.

Introduction

Protein fusion tags are essential tools for the isolation and purification of proteins for the study of protein:protein and protein:ligand interactions, function and structure. The most commonly used tag for purification and detection of recombinant expressed proteins is the polyhistidine tag (1). We recently developed an HQ fusion tag (HQHQHQ; H = histidine, Q = glutamine) that is similar to the polyhistidine tag in size. Both tags can be purified using immobilized metal affinity column purification (IMAC; 2). The HQ fusion tag, like the polyhistidine fusion tag, can be used as an N- or C-terminal fusion tag.

To address the need to purify polyhistidine- and HQ-tagged proteins in individual, small-volume columns, we have developed the HisLink™ Spin Protein Purification System[a,b,c], which uses the versatile HisLink™ Resin in a vacuum or centrifugation format. We have shown that the HisLink™ Protein Purification Resin[a,b] purifies polyhistidine-tagged proteins directly from various expression systems under native and denaturing conditions (2,3). The resin is a modified silica-based resin that provides a tetradentate metal-chelated solid support with a high binding capacity, and eliminates the nonspecific binding characteristic of unmodified silica. This resin was introduced for the purification of polyhistidine- and HQ-tagged proteins from large-volume batch or column purification systems (4). In this article we describe the applications and advantages of the HisLink™ Spin Purification System.

Using the HisLink™ Spin Protein Purification System

The HisLink™ Spin Protein Purification System purifies proteins from bacterial cell culture using either a tabletop centrifuge or a vacuum manifold as shown by the schematic, Figure 1. A mixture of FastBreak™ Cell Lysis Reagent[a,b,c], DNase I and HisLink™ Resin is added directly to 700µl of cell culture, resulting in efficient cell lysis and protein binding to the resin. After incubation, the lysate and resin are transferred to the spin columns for resin capture. Lysates flow easily through the spin columns, as the addition of DNase I eliminates clogging that might otherwise occur when using high-density cell cultures. Resin washes are performed using either a vacuum or a tabletop centrifuge. Finally, the protein of interest is eluted using a tabletop centrifuge. Similar amounts of protein are purified using either centrifugation or the vacuum method (Figure 2).

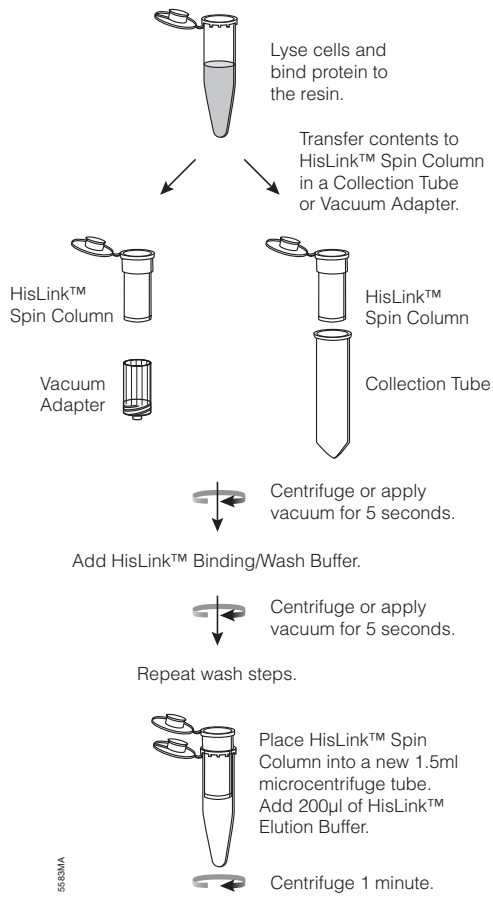

Figure 1. Schematic of the HisLink™ Spin Protein Purification System protocol.

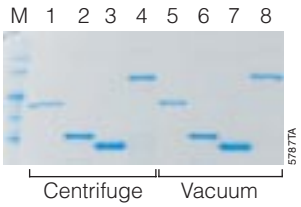

Figure 2. Comparison of vacuum and centrifuge methods for purification of polyhistidine- and HQ-tagged proteins from BL21(DE3) bacterial cells using the HisLink™ Spin Protein Purification System. Purified proteins were separated by electrophoresis on a 4–20% Tris-glycine gel (Invitrogen), stained with SimplyBlue™ SafeStain (Invitrogen) and destained with NANOpure® water. Lane M, Broad Range Protein Molecular Weight Markers (Cat.# V8491); lanes 1–4, purification by centrifugation method; lanes 5–8, purification by vacuum method; lanes 1 and 5, HQ-maltose binding protein; lanes 2 and 6, polyhistidine-GFP; lanes 3 and 7, HQ-chloramphenicol acetyltransferase (CAT); lanes 4 and 8, polyhistidine-firefly luciferase.

Optimization of Purification Methods

Many different polyhistidine- and HQ-tagged proteins can be purified using the HisLink™ Spin Protein Purification System (Figure 3). However, additional optimization of the purification method may be required depending on the particular protein. For example, some proteins form inclusion bodies, which require optimization of buffer conditions and additives for maximal yields. Chaotropic agents such as urea or guanidine-HCl will not affect binding or elution of polyhistidine-tagged proteins using the HisLink™ Resin (4). The HisLink™ Spin Protein Purification System is a useful system for optimizing multiple conditions from a single culture.

Scalable Protein Purification Methods

Because the HisLink™ Spin Protein Purification System uses the same resin chemistry as the HisLink™ Protein Purification Resin and the HisLink™ 96 Protein Purification System, the protocols are easily scalable. For example, a stringent wash method has been developed for processing a 700µl culture; this optimized protocol can be easily scaled for high-throughput automated systems using the HisLink™ 96 System (5) or for large volumes using the HisLink™ Resin.

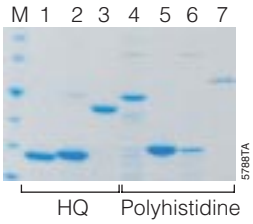

Figure 3. Purification of multiple polyhistidine- and HQ-tagged proteins directly from BL21(DE3) bacterial cell cultures using the HisLink™ Spin Protein Purification System. After purification with the HisLink™ Spin Protein Purification System using centrifugation, gel electrophoresis and staining was performed as described in the legend for Figure 2. Lane M, Broad Range Protein Molecular Weight Markers (Cat.# V8491); lane 1, HQ-chloramphenicol acetyltransferase (CAT); lane 2, HQ-glutathione transferase (GST); lane 3, HQ-maltose binding protein; lane 4, polyhistidine-MAPK; lane 5, polyhistidine-GFP; lane 6, polyhistidine-calmodulin; lane 7, polyhistidine-firefly luciferase.

Conclusion

The HisLink™ Spin Protein Purification System is a small-volume, single-tube, single-step protein purification system for use with a vacuum manifold or tabletop centrifuge. Using the high-binding capacity of HisLink™ Resin and a proprietary cell lysis reagent, polyhistidine- or HQ-tagged proteins can be purified under native, denatured or stringent binding and wash conditions. Cell lysis and protein binding occur simultaneously in a single step, followed by washing using either vacuum or centrifugation. The HisLink™ Spin Protein Purification System is a quick and easy purification system for small-volume, single-column needs, and is also scalable for purification of proteins from larger culture volumes or 96-well formats.

References

1. Yip, T.T. *et al.* (1989) *Anal. Biochem.* **183**, 159–71.
2. Godat, B. *et al.* (2005) *Promega Notes* **91**, 17–20.
3. Engel, L. *et al.* (2005) *Promega Notes* **90**, 15–8.
4. Simpson, D. and Godat, B. (2004) *Promega Notes* **86**, 11–4.
5. *Automated HisLink™ 96 Protein Purification System Automated Protocol* #EP028, Promega Corporation.

Protocols

- *HisLink™ Spin Protein Purification System Technical Manual* #TM281, Promega Corporation. www.promega.com/tbs/tm281/tm281.html
- *HisLink™ Protein Purification Resin Technical Bulletin* #TB327, Promega Corporation. www.promega.com/tbs/tb327/tb327.html
- *HisLink™ 96 Protein Purification System Technical Bulletin* #TB342, Promega Corporation. www.promega.com/tbs/tb342/tb342.html
- *HisLink™ 96 Protein Purification System Automated Protocol* #EP028, Promega Corporation. www.promega.com/tbs/ep028/ep028.html

Ordering Information

Product	Size	Cat.#
HisLink™ Spin Protein Purification System	25 reactions	V1320
HisLink™ Protein Purification Resin	50ml	V8821
	5ml	V8823
HisLink™ 96 Protein Purification System	1 × 96	V3680
	5 × 96	V3681

Related Products

Product	Size	Cat.#
Broad Range Protein Molecular Weight Markers	100 lanes	V8491
Vac-Man® Laboratory Vacuum Manifold	1 each	A7231

(a) Patent Pending.

(b) Certain applications of this product may be covered by patents issued to parties other than Promega and applicable in certain countries. Because purchase of this product does not include a license to perform any of these patented applications, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used.

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Related Citation

Teghanemt, A. *et al.* (2005) Molecular basis of reduced potency of underacylated endotoxins. *J. Immunol.* 175, 4669–76.

The role of various *Neisseria meningitidis* serotype B [NMB lipooligosaccharides (LOS)] in TLR4-dependent cell activation was investigated. A known protein binding partner, myeloid differentiation protein 2 (MD-2), was expressed in a baculovirus system with a 6X-polyhistidine tag and the insect medium incubated with purified hexa-, penta- and tetra-acylated endotoxins metabolically labeled with [^{3}H]- or [^{14}C]acetate. These complexes (containing 0.2–1ng of radiolabeled LOS) were then incubated for 1 hour at 25°C with 100µl of HisLink™ Protein Purification Resin. After incubation, the resin was centrifuged, the supernatant removed and the resin washed 3–4 times in 500µl of PBS containing 5mM imidazole before elution with 2% SDS. The presence of radiolabeled LOS was evaluated by liquid scintillation spectroscopy and results were expressed as the percentage of LOS captured.

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