

MagneHis™ Protein Purification System: Purification of His-Tagged Proteins in Multiple Formats

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Abstract

The MagneHis™ Protein Purification System provides a fast and efficient method for purifying His-tagged proteins with high yield and low background. The flexible protocol can be used for small- or large-scale purification and can be performed manually or using a robotic platform. The proprietary MagneHis™ Cell Lysis Reagent makes cell lysis quick and simple.

MagneHis™ Cell Lysis Reagent minimizes unnecessary handling and reduces the time required for cell lysis.

Introduction

Production of recombinant proteins using expression systems is a common laboratory technique. Proteins synthesized in such a manner can be used for several different applications, including enzymatic assays (1–3), studying intermolecular relationships (e.g., protein:protein interactions, nucleic acid:protein interactions; 1,4–6) or structural studies (e.g., X-ray crystallography, NMR; 7,8).

Fusion tags are small stretches of amino acids added to the N- or C-terminus of a recombinant protein. The inclusion of fusion tags into specific expression vectors facilitates recombinant protein purification and detection. Certain tags are used because they encode an epitope that can be purified or detected by a specific antibody, or they have distinct physical characteristics (e.g., ligand binding) that can be used in a purification format.

The affinity of histidine residues for immobilized nickel allows for the selective purification of proteins that contain histidine residues (9,10). This observation led to the design of specific cloning vectors that contain 5–10 histidine residues at either the C- or N-terminus of the expressed protein. The use of a His tag has several advantages over other tags:

- 6X His tags add only 0.84kDa to the mass of the protein, while GST or protein A add 26 or 30kDa, respectively.
- The small histidine tail is not immunogenic; thus, there is no requirement for its removal prior to injection into an animal for antibody production.
- Tertiary structure is not important for purification; therefore, His-tagged proteins can be isolated using denaturing conditions.

There are four primary criteria required for the successful purification of expressed His-tagged proteins: 1) efficient cell lysis; 2) minimal nonspecific binding; 3) maximal binding of the His-tagged protein to the nickel substrate; 4) efficient elution of the bound protein.

The MagneHis™ Protein Purification System^(a,b) successfully meets all of these criteria.

General Procedure For Purifying His-Tagged Proteins

A typical procedure for the purification of a His-tagged protein involves four basic steps:

Cell Lysis. This can be accomplished by nonenzymatic methods (e.g., sonication or French Press) or by using hydrolytic enzymes such as lysozyme.

Binding to Ni-Particles. After cell lysis, cellular debris is removed by centrifugation. The appropriate amount of Ni-particles is added to the supernatant, or the supernatant is added to a column of Ni-particles.

Washing. Once the His-tagged protein has been bound, any unbound proteins are washed away by 2–3 washing steps.

Elution. There are two methods for the elution of bound His-tagged proteins. The first method uses an elevated concentration of imidazole at a constant pH to displace the histidine tail from the Ni-particles. The second method uses low pH. At low pH (< 4.5), histidines are protonated and do not interact with the Ni-particles.

The MagneHis™ Protein Purification System (Figure 1) optimizes these steps.

High Yield With Little or No Nonspecific Background

Most commercial systems have a protein binding capacity of only 300µg/ml of Ni-particles. Promega's system has a binding capacity of up to 1mg/ml of MagneHis™ Ni-Particles. The high binding capacity provides an economical method for purifying His-tagged proteins.

Another critical aspect of His-tagged protein purification is minimizing the binding of nonspecific proteins. This is especially important for the purification of His-tagged proteins that express poorly. Figure 2 illustrates the purification of a His-tagged RNase H1 that expresses poorly. Even at these low expression levels, the MagneHis™ Ni-particles were able to facilitate purification with minimal nonspecific background.

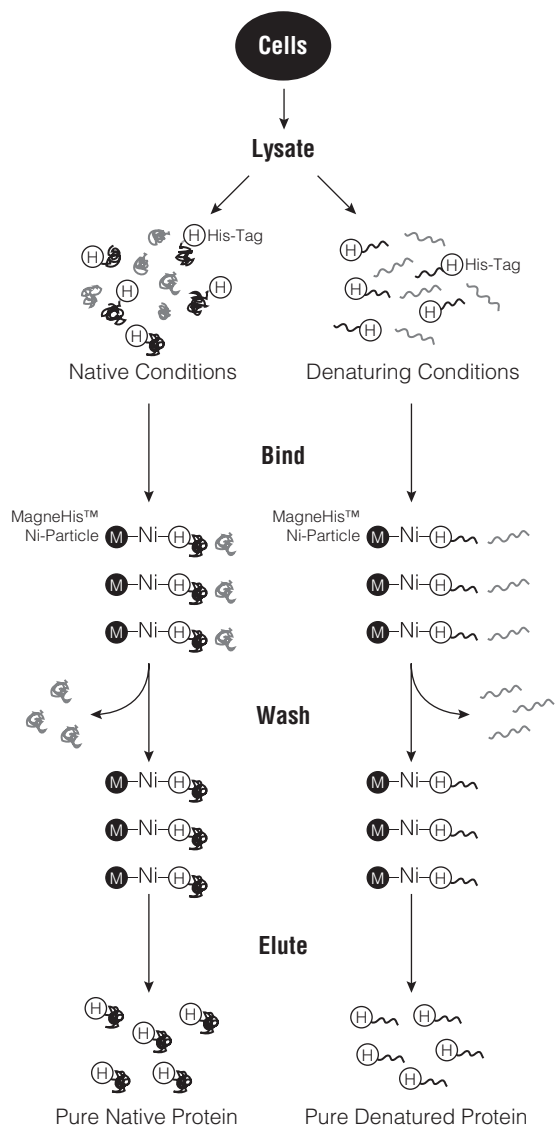


Figure 1. Diagram of the MagneHis™ Protein Purification System.

Each production batch of the MagneHis™ Ni-Particles is evaluated for nonspecific protein binding. A quality control specification has been established using an *E. coli* lysate that does not contain a His-tagged protein (Figure 3). Nonspecific binding in the assay is <10%.

A Rapid Method for Analyzing and Purifying His-Tagged Proteins

When handling multiple samples, the use of sonication or a French Press to lyse samples can be cumbersome. Lysozyme incubation adds 30 minutes to the lysis procedure. Promega's MagneHis™ Cell Lysis Reagent minimizes unnecessary handling and reduces the time required for cell lysis. Cells are briefly centrifuged, and the pellet is resuspended with the MagneHis™ Cell Lysis Reagent. The cells are lysed during a 10-minute incubation.

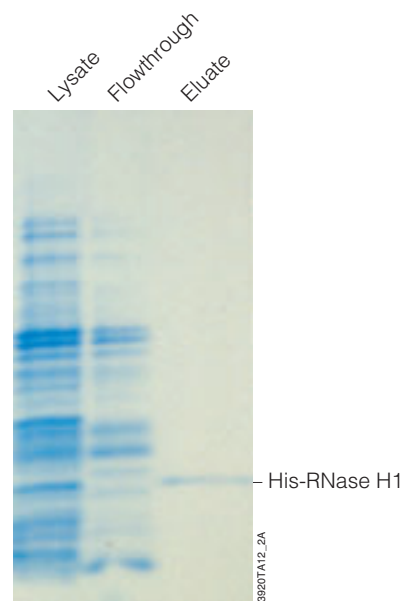


Figure 2. Purification of His-tagged RNase H1 expressed at a low level.

A cell culture expressing a His-tagged RNase H1 was centrifuged at $13,000 \times g$ for 10 minutes. The supernatant was removed, and the pellet was frozen at -70°C . The pellet was resuspended in MagneHis™ Cell Lysis Reagent and incubated for 10 minutes at room temperature with shaking. The sample was centrifuged at $13,000 \times g$ for 10 minutes, and 200 μl of the supernatant was added to 30 μl MagneHis™ Ni-Particles. The sample was mixed by pipetting and incubated for two minutes at room temperature. The sample was placed in a magnetic stand, and the flowthrough was collected. The MagneHis™ Ni-Particles were washed three times with MagneHis™ Binding/Wash Buffer containing 100mM HEPES, 10mM imidazole (pH 7.5). The His-tagged RNase H1 was eluted with 100 μl of MagneHis™ Elution Buffer (100mM HEPES, 500mM imidazole [pH 7.5]). Samples were analyzed by SDS-PAGE and visualized by GelCode Blue staining.

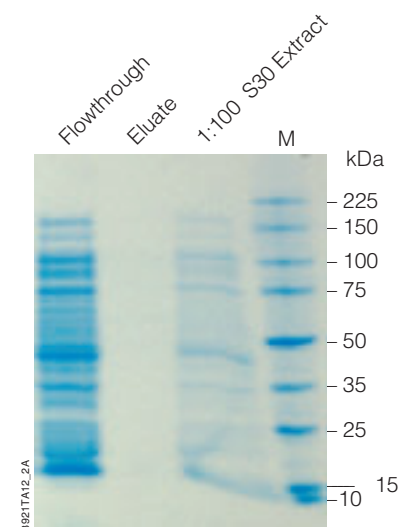


Figure 3. Minimal nonspecific binding of proteins to MagneHis™ Ni-Particles.

E. coli S30 Extract was diluted 1:10 with MagneHis™ Binding/Wash Buffer, and 100 μl was added to 30 μl MagneHis™ Ni-Particles. MagneHis™ purification was performed as described in Figure 2. Samples were analyzed by SDS-PAGE and visualized by GelCode Blue staining.

Attractive Protein Purification... continued

After cell lysis, most procedures recommend that cellular debris be removed before binding the protein to the Ni-particles. This can increase the time required, especially if several samples are being processed at once. Using the MagneHis™ Cell Lysis Reagent, there is usually no need to remove cellular debris prior to adding the MagneHis™ Ni-Particles (Figure 4).

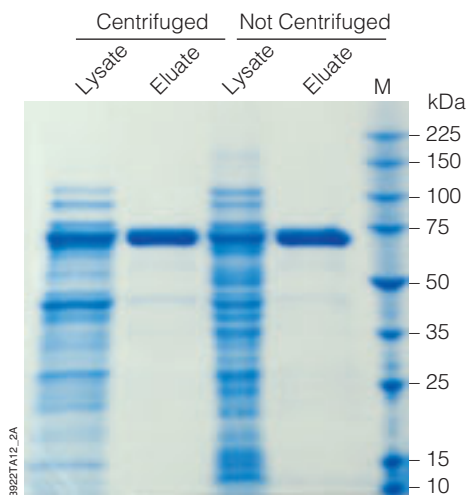


Figure 4. Purification by centrifugation vs. no centrifugation. MagneHis™ Cell Lysis Reagent was added to pelleted cells expressing a His-tagged methionyl tRNA synthetase and was incubated for 10 minutes at room temperature with shaking. The lysate was either used directly for MagneHis™ purification or was cleared of cellular debris by centrifugation and the supernatant was used for MagneHis™ purification. MagneHis™ purification was performed as described in Figure 2. Samples were analyzed by SDS-PAGE and visualized by GelCode Blue staining.

Large-Scale Purification of His-Tagged Proteins

In order to characterize a recombinant His-tagged protein, typically several clones are initially screened on a small scale (e.g., 1ml cultures) to identify the clone that exhibits optimal expression. Further optimization of expression is sometimes required on a small scale (e.g., changing the host strain or growth conditions).

Usually, column-based methods are used for the large-scale purification of His-tagged proteins intended for structural or functional studies. Purification by column methods can take several hours. The MagneHis™ Protein Purification System can purify proteins from a 1L culture in less than 30 minutes. Figure 5 shows that the MagneHis™ System can be used in both small- and large-scale formats without altering the basic procedure.

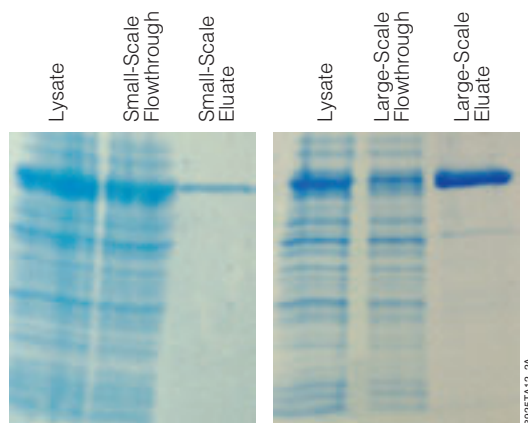


Figure 5. Small- vs. large-scale purification. A 100ml culture expressing His-tagged methionyl tRNA synthetase was centrifuged at 6,000rpm for 15 minutes. The supernatant was discarded, and the pellet was frozen at -70°C . The pellet was resuspended in 10ml of MagneHis™ Cell Lysis Reagent and incubated for 10 minutes at room temperature with shaking. The supernatant was used to compare small- and large-scale His-tagged purification.

Small scale: The purification was performed as described in Figure 2 using 100 μl of supernatant added to 30 μl MagneHis™ Ni-Particles.

Large scale: 8ml of lysate was added to 2.4ml MagneHis™ Ni-Particles in a conical tube and was mixed by inverting the tube for two minutes at room temperature, then placed in a magnetic stand. The flowthrough was poured off, and an aliquot was collected. The MagneHis™ Ni-Particles were washed three times with 12ml of MagneHis™ Binding/Wash Buffer containing 100mM HEPES, 10mM imidazole (pH 7.5). The His-tagged protein was eluted with 800 μl of MagneHis™ Elution Buffer (100mM HEPES, 500mM imidazole [pH 7.5]). Samples (200 μl) were analyzed by SDS-PAGE and visualized by GelCode Blue staining.

Advantages of the MagneHis™ Cell Lysis Reagent

The MagneHis™ Cell Lysis Reagent provides a fast and efficient alternative to time-consuming lysozyme incubations for bacterial cell lysis. This proprietary formulation provides maximum release of cellular proteins in 10 minutes, streamlining a critical incubation step in high-throughput applications. The extent of cell lysis is comparable with standalone lysis reagents. Figure 6A compares the total protein released from bacterial cell lysis using MagneHis™ Cell Lysis Reagent and two other commercial products. The MagneHis™ Cell Lysis Reagent has an added benefit of potentially stabilizing protein structure and thus retaining higher levels of enzyme activity. This is demonstrated in the cellular lysis of a clone expressing firefly luciferase (Figure 6B). This stabilizing effect has also been observed for β -galactosidase and *Renilla* luciferase (data not shown).

Automated Purification of His-Tagged Proteins

As the field of proteomics requires products that can be used on a high-throughput scale, the requirement for performing protocols on robotic platforms increases. The MagneHis™ Protein Purification System is designed to be used both at the bench for single purifications as well as on various robotic platforms. For more details regarding high-throughput applications, refer to the accompanying article in this issue (11).

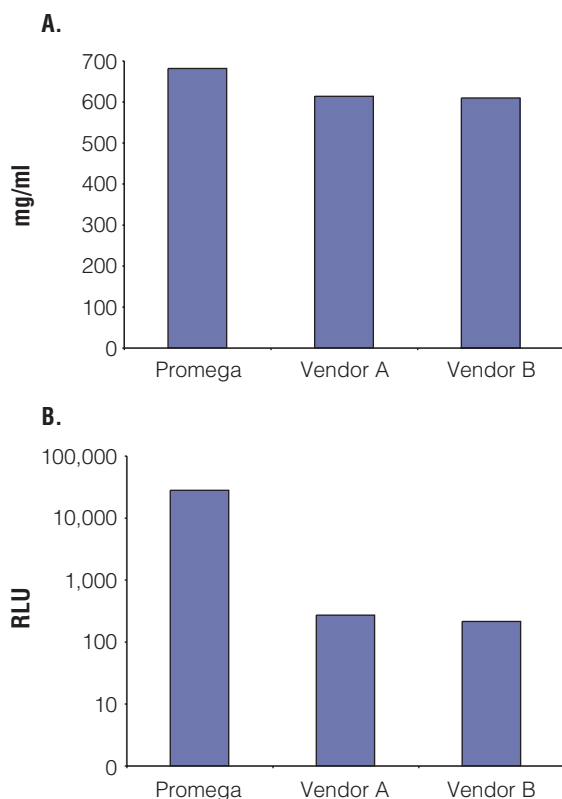


Figure 6. Comparison of the MagneHis™ Cell Lysis Reagent to commercially available lysis reagents. Cells expressing 6X His-tagged firefly luciferase were lysed using either MagneHis™ Cell Lysis Reagent, Vendor A or Vendor B reagents following the respective manufacturers' protocols. Lysates were assayed for protein concentration by BCA assay (**Panel A**) and luciferase activity (**Panel B**).

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Protocols

- ◆ *MagneHis™ Protein Purification System Technical Manual #TM060*, Promega Corporation. (www.promega.com/tbs/tm060/tm060.html)

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Ordering Information

Product	Size	Cat.#
MagneHis™ Protein ^(a,b) Purification System	2ml	V8500
	10ml	V8550
	12 × 75mm	Z5333
MagneHis™ Ni-Particles ^(a,b)	2ml	V8560
	10ml	V8565
Magnetic Separation Stand (two position)	0.5ml	Z5331
	1.5ml	Z5332
	12 × 75mm	Z5333
MagneSphere® Technology Magnetic Separation Stand (twelve position)	0.5ml	Z5341
	1.5ml	Z5342
	12 × 75mm	Z5343
PolyATtract® System 1000 Magnetic Separation Stand	1 each	Z5410
MagneSphere® Magnetic Separation Stand, 24 well	1 each	Z5441
MagneSphere® Magnetic Separation Stand, 96 well	1 each	Z5431

^(a) Patent Pending.

^(b) Certain applications of this product are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, 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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