FINDING THE RIGHT PROTEIN PURIFICATION SYSTEM

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Researchers often need to purify a single protein for further detailed study. One method for isolating a target protein involves affinity purification. A recombinant fusion protein that is "tagged" with a stretch of amino acids or other small protein is expressed in bacteria. The fusion protein is purified based on specific biochemical properties that are conferred by the tag. Here we discuss factors that influence the choice of protein tag and purification system.

Introduction

Information about the regulation of protein expression, protein modification, protein:protein interactions and protein function during different stages of cell development is needed to understand the development and physiology of organisms. Thus, developing tools for studying proteins is critical. Protein purification is a fundamental step for studying individual proteins, and a variety of strategies for purifying proteins have been developed to address desired scale, throughput and downstream applications.

Tagging a Protein for Affinity Purification: His-Tag and GST-Fusion Systems

Affinity purification tags can be fused to any protein of interest, allowing fast and easy purification following a procedure that is based on the affinity properties of the tag (1). 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.

Promega protein purification systems allow users to choose their desired format and tag, depending on their experimental goals.

Certain tags are used because they encode an epitope that can be purified or detected by a specific antibody or because they enable simplified purification of the desired protein. The biochemical features of different tags influence the stability, solubility and expression of the proteins to which they are attached (2). Among these tags, the His-tag (polyhistidine) is the most widely used and has several advantages (3). The small size renders a His-tag less immunogenic than other larger tags. His-tags may not need to be removed for downstream applications with the purified protein. Second, a large number of commercial vectors for expressing His-tagged proteins are available to researchers. Additionally, the tag may be placed at either the N- or C-terminus. And finally, the interaction of the His-tag does not depend on the tag structure, making it possible to purify otherwise insoluble proteins using denaturing conditions.

Protein purification with a glutathione-S-transferase (GST) affinity tag was introduced in 1988 by Smith and Johnson (4) and is based on the strong affinity of GST for glutathione-covered matrices. Glutathione-S-transferases are a family of cytosolic proteins with multiple functions that are present in eukaryotic organisms (5,6). GST isoforms are not normally found in bacteria, thus there is no competition from endogenous bacterial proteins for binding to purification resin with GST-fusion proteins expressed in bacteria. The GST purification tag discussed here is a 26kDa protein from the parasite *Schistosoma japonicum*. GST-fusion protein binding to glutathione is reversible and allows efficient elution of the bound GST-fusion protein with free glutathione.

Purifying an Expressed Fusion Protein

There are four basic steps required to purify an expressed fusion protein:

- **Cell Lysis.** This can be accomplished by nonenzymatic methods (e.g., sonication or French Press) or by using hydrolytic enzymes such as lysozyme or a detergent reagent such as FastBreak™ Cell Lysis Reagent[a,b].
- **Binding to Matrix.** After cell lysis, cellular debris is removed by centrifugation. The appropriate amount of binding matrix is added to the supernatant, or the supernatant is added to a column of matrix.
- **Washing.** Once the fusion protein has been bound, any unbound proteins are washed away by 2–3 washing steps.
- **Elution.** When using a His-based matrix, a high concentration of imidazole at a constant pH is used to displace the histidine tail from the Ni-particles, or with the GST-based system, reduced glutathione is added.

Rapidly Purifying Proteins on a Small Scale

To address the need for fast, convenient purification that is amenable to high-throughput screening, we developed magnetic formats for purifying either 6X His-tagged proteins [the MagneHis™ Protein Purification System[a] (Figure 1)] or GST-tagged proteins [MagneGST™ Protein Purification System[c] (Figure 2)]. The particles enable protein purification without multiple centrifugation steps or transfer of samples to multiple tubes, which is required when nonmagnetic resins are used for batch purification of proteins. When handling multiple

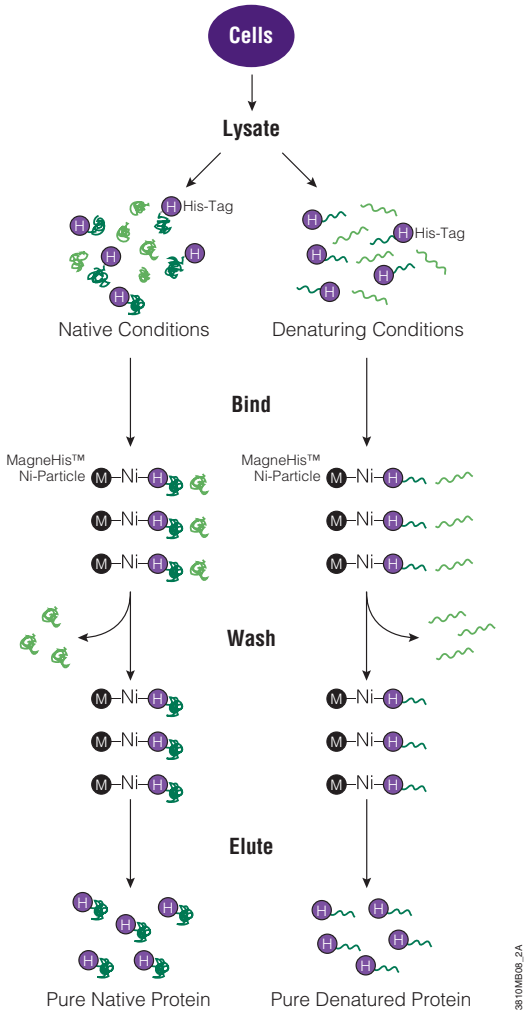

Figure 1. Diagram of the MagneHis™ Protein Purification System protocol. Protocol requires a magnetic stand.

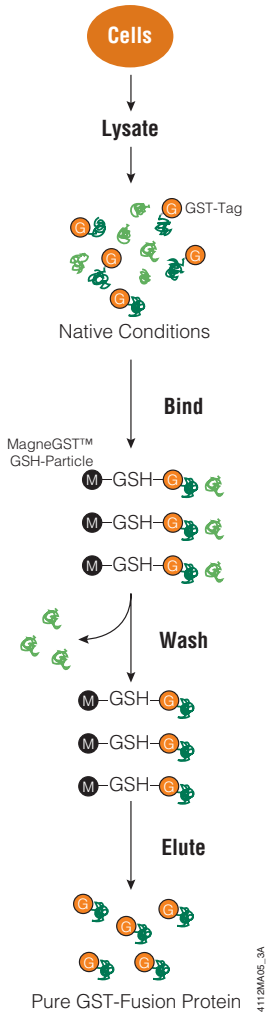

Figure 2. Diagram of the MagneGST™ Protein Purification System protocol. Protocol requires a magnetic stand.

samples, using sonication or a French Press to lyse samples can be cumbersome. Lysozyme incubation adds 30 minutes to the lysis procedure. Both the MagneHis™ and the MagneGST™ Systems provide a Cell Lysis Reagent that minimizes unnecessary handling and reduces the time required for cell lysis. Cells are briefly centrifuged, the pellet is resuspended and the cells are lysed during a 10-minute incubation.

After cell lysis, most procedures involving proteins recommend that cellular debris be removed before binding the protein to the affinity matrix. This can increase the time required, especially if several samples are being processed at once. Using the provided Cell Lysis Reagent, there is no need to remove cellular debris prior to adding either of the magnetic particles (Figure 3).

Purifying Proteins on a Large Scale

Larger amounts of fusion proteins are required for many applications, including enzymatic assays (7–9) and structural studies (e.g., X-ray crystallography, NMR; 10,11). The HisLink™ Protein Purification Resin provides a conventional means to purify large amounts of His-tagged proteins in a variety of formats, including gravity-column, batch or FPLC.

One of the primary advantages of the HisLink™ Resin is the ability to use it for batch purification of protein from crude lysate. In batch mode, the protein of interest is bound to the resin by mixing lysate with the resin for approximately 30 minutes. Once bound with protein, the resin is allowed to settle to the bottom of the container, and the spent lysate is decanted. The particles are washed, and then the wash buffer is carefully decanted. Final elution is best achieved by transferring the HisLink™ Resin to a column for elution of the protein in fractions. The advantages of batch purification are: 1) Less time is required to perform the purification; 2) Large amounts of lysate can be processed; and 3) Clearing the lysate prior to purificaton is not required. Figure 4 shows proteins purified with HisLink™ Resin using gravity-flow column and batch methods with and without clearing. The resulting

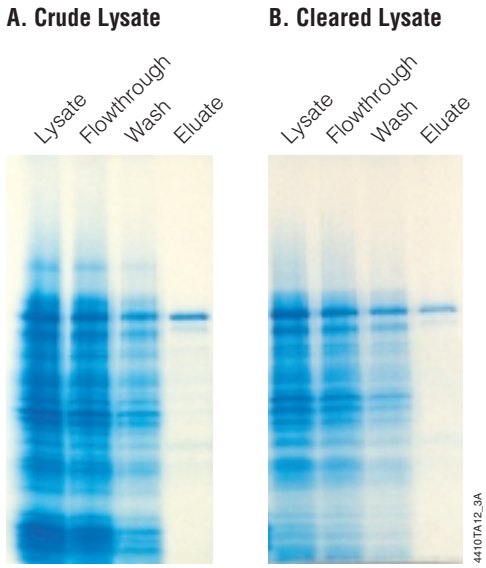

Figure 3. Purification of expressed protein using MagneGST™ Particles. Bacterial cultures (1ml) were pelleted and lysed. One lysate was added directly to 100µl of MagneGST™ Particles without removing cell debris (**Panel A**). The other lysate was cleared by centrifugation at 12,000 × *g* for 40 minutes before adding 100µl of MagneGST™ Particles (**Panel B**). Both samples were then processed according the MagneGST™ System protocol (Technical Manual #TM240).

quality and the amount of purified protein was independent of the method used for purification.

Automating Protein Purification

While it is possible to process samples faster manually than with the Biomek® 2000 or Tecan Genesis® RSP workstations, manual processing of 96 samples at a time is labor intensive, tedious and prone to error. We have automated both the MagneHis™ and MagneGST™ Protein Purification Systems for rapid purification of proteins on several automated workstations including the Biomek® 2000, Biomek® FX, Tecan Genesis® RSP and KingFisher® automated platforms (Figures 5 and 6). These methods allow a quick startup in your laboratory and offer several key advantages for end-users. Advantages include a simple process that is easily automated on a variety of platforms for high-throughput applications, chemistry that is easily scaled-up for larger sample volumes and high-quality purified proteins with few background bands.

As the need for high-throughput protein purification increases, techniques limiting the number of samples that can be handled efficiently are not adequate. To meet the need for a lysis method compatible with high-throughput processing, we developed the FastBreak™ Cell Lysis Reagent. This reagent is designed for efficient, gentle lysis of *E. coli* cultures without centrifugation or mechanical cell disruption. The reagent is provided as a 10X concentrate and contains a proprietary nonionic detergent to facilitate lysis. The reagent is added

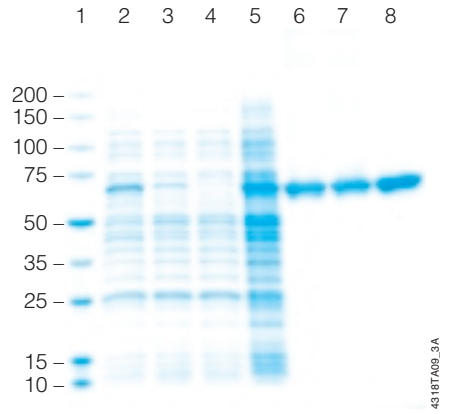

Figure 4. Purification of 6X His-tagged fusion protein using HisLink™ Resin in a column and batch format with both cleared and crude lysate. Lane 1, Broad Range Protein Molecular Weight Markers (Cat.# V8491); lane 2, bacterial lysate expressing 6X His-tagged protein; lane 3, flowthrough of cleared lysate (column); lane 4, flowthrough of cleared lysate (batch); lane 5, flowthrough of crude lysate (batch); lane 6, elution of 6X His-tagged protein from cleared lysate (column); lane 7, elution of 6X His-tagged protein from cleared lysate (batch); lane 8, elution of 6X His-tagged protein from crude lysate (batch).

directly to *E. coli* culture; the cells are disrupted, and protein is released during a brief incubation. Recombinant proteins can be directly screened in the resulting extract or purified by the addition of an appropriate affinity matrix (i.e., MagneHis™ Protein Purification System). This format allows users to perform the procedure manually or on a robotic platform, such as the Biomek® 2000 or FX workstation, allowing high-throughput applications.

Analyzing Proteins Using Mass Spectrometry

The methods normally used for His-tagged protein purification result in the presence of imidazole and high concentrations of salt and detergents that interfere with mass spectrometric analysis by causing high backgrounds. However, modified washing and elution conditions can decrease these high backgrounds for His-tagged proteins. The method we have developed for purifying His-tagged proteins using the MagneHis™ Protein Purification System can drastically reduce the background levels in MALDI-TOF mass spectrometry analysis (See Technical Manual #TM060 for more information).

Summary

Purifying recombinant fusion proteins is an essential step for many applications, including generating antibodies for localization, studying protein biochemistry, determining protein:protein interactions, and assaying enzyme activity in vitro. Promega protein purification systems allow users to choose their desired format and tag, depending on their experimental goals. ■

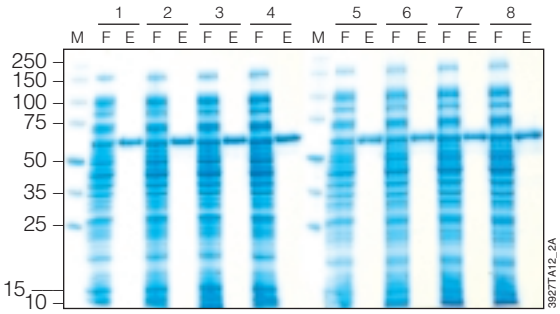

Figure 5. Automated protein purification of His-tagged thermostable firefly luciferase on the KingFisher® workstation using MagneHis™ Protein Purification System. Eight samples of bacteria expressing His-tagged thermostable firefly luciferase were cultured. For each sample, purified protein was eluted from the MagneHis™ Ni-Particles (lanes E), and the flowthrough fractions of proteins not captured by the MagneHis™ Ni-Particles were collected (lanes F). Lane M contains the Broad Range Protein Molecular Weight Markers (Cat.# V8491).

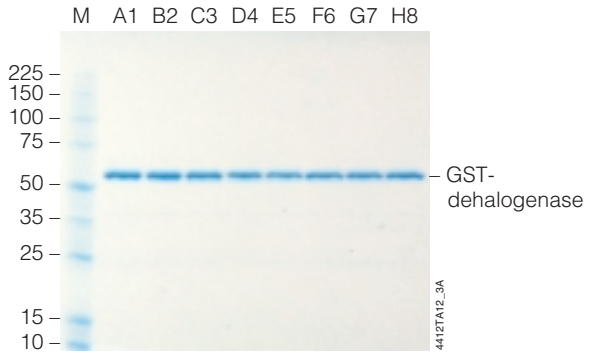

Figure 6. Automated protein purification of GST-dehalogenase fusion protein using MagneGST™ purification resin. GST-dehalogenase was purified from 1ml bacterial cultures in a 96-well format on the Beckman Biomek® 2000 using MagneGST™ Particles and the protocol outlined in Figure 2. Random samples were selected, and a portion of the eluates (2.5%) was analyzed by SDS-PAGE. These data demonstrate consistent yields across the plate. Lane M contains the Broad Range Protein Molecular Weight Markers (Cat.# V8491).

References

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Protocols

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

MagneGST™ Protein Purification System Technical Manual, #TM240 Promega Corporation (www.promega.com/tbs/tm240/tm240.html)

HisLink™ Protein Purification Resin Technical Bulletin, #TB327, Promega Corporation (www.promega.com/tbs/tb327/tb327.html)

FastBreak™ Cell Lysis Reagent, 10X Product Information #9PIV857, Promega Corporation (www.promega.com/tbs/9PIV857/9PIV857.html)

Ordering Information

Product	Size	Cat.#
MagneHis™ Protein Purification System[a]	65 reactions	V8500
	325 reactions	V8550
MagneHis™ Ni-Particles[a]	2ml	V8560
	10ml	V8565
MagneGST™ Protein Purification System[c]	40 reactions	V8600
	200 reactions	V8603
MagneGST™ Glutathione Particles[c]	4ml	V8611
	20ml	V8612
HisLink™ Protein Purification Resin	50ml	V8821
FastBreak™ Cell Lysis Reagent, 10X[a,b]	10ml	V8571
	40ml	V8572
	100ml	V8573
MagneSphere® Technology Magnetic Separation Stand	12-position; 1.5ml tubes	Z5342
	12-position; 75mm tubes	Z5343
	2-position; 1.5ml tubes	Z5332
	2-position; 75mm tubes	Z5333
MagneSil® Magnetic Separation Unit	conical tube	A2231

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