Purification of GST-Fusion Proteins by Magnetic Resin-Based MagneGST™ Particles

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The MagneGST™ Protein Purification System provides a simple, rapid and reliable method for the purification of glutathione-S-transferase (GST) fusion proteins with high efficiency and low background. The magnetic resin-based batch purification method eliminates the need for multiple centrifugation cycles and provides a simple purification that can be performed manually or on a robotic platform. Purification of the GST-fusion protein starting from cell pellets can be accomplished in less than 2 hours from either crude or cleared lysates.

MagneGST™ Glutathione Particles fill the need for a quick, convenient protein purification tool that can be used in high-throughput screening.

Introduction

Elucidation of protein function is one of the major tasks facing researchers today. 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 various organisms. Thus development of tools to enhance the study of proteins is critical. In recent years, many tools have been developed to study individual proteins. Currently, however, efforts are directed towards the study of proteins on a high-throughput scale, as the ability to rapidly express and isolate hundreds of different proteins becomes increasingly important.

Affinity purification tags can be fused to any protein of interest, allowing fast, easy purification, following a procedure based on the affinity properties of the tag (1). Various affinity purrification tags are currently used for protein purification and detection. Different tags have different features that influence the stability, solubility and expression of the proteins they're attached to in bacteria (2). In a recent report a large set of randomly selected human cDNAs were cloned and expressed as fusion proteins with 4 different tags, then characterized for yield, purity and stability (3). In this study and others, a GST affinity purification tag was shown to enhance solubility of many eukaryotic proteins expressed in bacteria.

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. GST-fusion protein binding to glutathione is reversible, allowing efficient elution of the bound GST-fusion protein by addition of reduced glutathione to the elution buffer. Mild elution conditions allow for the use of the purified protein in downstream applications of GST-fusion protein without the need for dialysis.

Glutathione-S-transferases are a family of cytosolic proteins present in eukaryotic organisms that have multiple functions (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 used for this report is a 26kDa protein from the parasite *Schistosoma japonicum* (4).

To address the need for fast, convenient purification that is amenable to high-throughput screening we developed a magnetic GST purification resin, MagneGST™ Glutathione Particles[a] (Cat.# V8611). The particles enable GST protein purification without the need for multiple centrifugation steps and transfer of samples to multiple tubes, which is required when nonmagnetic resins are used for batch purification of proteins.

There are several criteria that define a good protein purification resin: minimal nonspecific protein binding, high binding capacity for the fusion protein and efficient recovery of the fusion protein. In this report we show that the MagneGST™ Glutathione Particles meet these criteria, enabling purification of proteins with a broad range of molecular weights, at different expression levels. Furthermore, MagneGST™ Particles can be used on automated liquid-handling platforms for convenient walk-away purification.

Procedure for Purifying GST-Fusion Proteins

There are four basic steps involved in GST-fusion protein purification using MagneGST™ Particles as described in Figure 1. We have optimized these steps for efficient recovery of pure GST-fusion proteins. The magnetic nature of the resin allows purification to be performed in a single tube, thus simplifying and shortening the purification process.

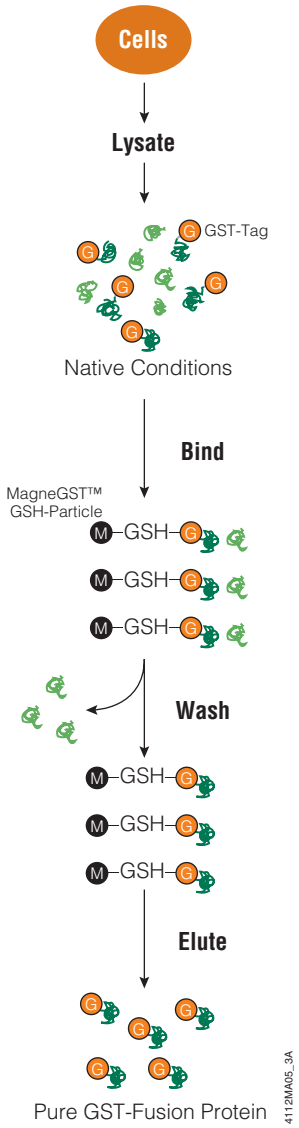

Figure 1. Schematic diagram of the MagneGST™ Protein Purification System. An induced bacterial culture expressing GST-fusion proteins is pelleted and lysed by enzymatic or mechanical methods. MagneGST™ Glutathione Particles are added directly to cleared or crude lysate. GST-fusion proteins bind to the particles during incubation at room temperature or at 4°C, then are washed to remove unbound and nonspecifically bound proteins; three wash steps are performed. GST-fusion protein is eluted from the particles by addition of 10–50mM reduced glutathione at pH 8.

Recovery of Pure GST-Fusion Protein with High Efficiency

A critical aspect of protein purification is the recovery of protein with little or no contaminants. Removal of nonspecifically bound proteins is especially important when purifying proteins that are poorly induced. Figure 2, Panel A, shows purification of a GST-fusion protein expressed at low levels. The protein purified with good yield and purity, indicating low nonspecific binding. In Figure 2, Panel B, we examined nonspecific binding of MagneGST™ resin in the absence of overexpressed GST-fusion protein. Panel B shows that even without competition there is virtually no nonspecific binding of protein to the resin, as demonstrated by the absence of S30 extract proteins in the eluted fraction (Figure 2, Panel B).

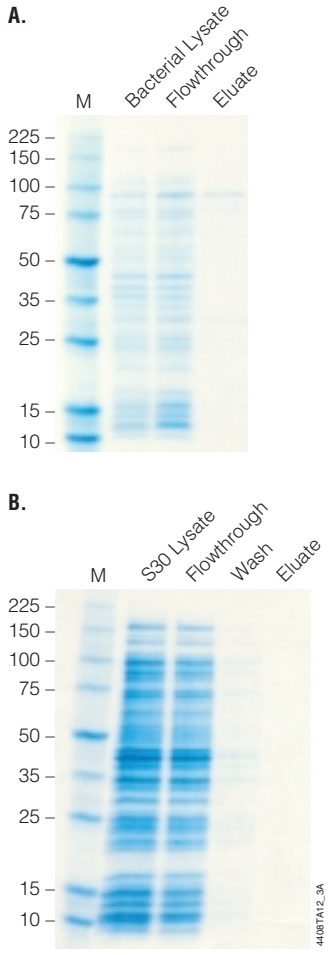

Figure 2. MagneGST™ Particles allow purification of low-expressing protein, with minimal nonspecific binding. Panel A. Crude bacterial lysate prepared from 1ml of culture expressing a GST-luciferase fusion protein. The fusion protein was purified according to the protocol outlined in Figure 1 using 100µl of MagneGST™ Particles. **Panel B.** Minimal nonspecific binding of proteins to MagneGST™ Particles using *E. coli* S30 Extract (30µl) and 100µl of MagneGST™ Particles. All samples were analyzed by SDS-PAGE and visualized with Coomassie® blue staining. Lanes M: Broad Range Protein Molecular Weight Markers (Cat.# V8491).

Another important feature of purification resin is its binding capacity. We estimate the binding capacity of the MagneGST™ Particles for GST-fusion protein to be 7mg of protein per mililiter of settled resin, much higher than that of another commercially available magnetic purification resin (capacity estimated at 2mg/ml of settled resin). The binding capacity of GST-fusion protein is comparable to that of nonmagnetic GST purification resins. Thus MagneGST™ resin provides an economical and effective way to recover GST-fusion proteins with high yield and purity.

Rapid Purification of GST-Fusion Proteins from Crude Bacterial Lysate

When purifying proteins from multiple samples simultaneously, the use of a French press or sonication for cell lysis can be cumbersome and time consuming. The MagneGST™ Purification System includes Cell Lysis Reagent for quick, convenient lysis. Induced bacterial culture is pelleted by brief centrifugation and pellets are resuspended in MagneGST™ Cell Lysis Reagent, followed by a 20- to 30-minute incubation.

In traditional protein purification systems using column or batch purification with GST-tagged protein, cellular debris must be removed by high-speed centrifugation, a process known as "clearing the lysate", before adding GST binding resin. This step is time consuming and creates a bottleneck when purifying proteins on automated platforms. MagneGST™ Glutathione Particles can be added directly to the crude cell lysate, eliminating the need for lysate clearing and providing a faster, more efficient procedure. Figure 3 shows proteins purified from crude or cleared lysate with the MagneGST™ System. The proteins obtained by either procedure were of equal purity and comparable yield.

During robotic purification of proteins from the crude lysate of strain BL21(DE3)pLysS grown to high cell density, we occasionally noticed contaminating proteins copurifying with large GST-fusion proteins. This is due to the release of nucleic acids from lysed cells, which increases sample viscosity and interferes with protein purification. When we added RQ1 RNase-Free DNase (Cat.# M6101) to the crude lysate, the purity of the GST-fusion proteins increased significantly (data not shown). The MagneGST™ System contains RQ1 RNase-Free DNase; the addition of the DNase during purification is optional but may aid purification when lysate viscosity is high.

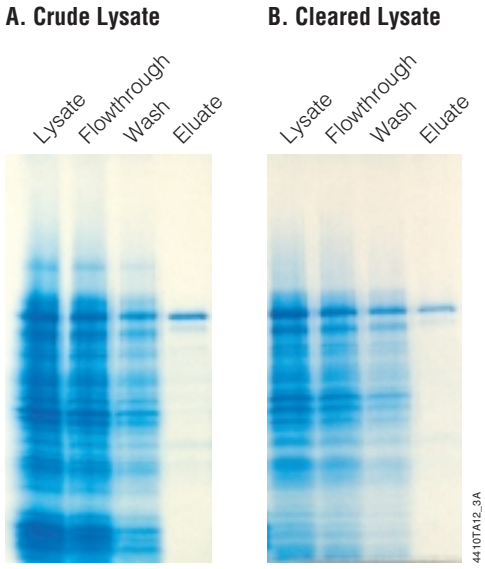

Figure 3. Purification from crude and cleared bacterial lysate. Bacterial cultures (1ml) were pelleted and lysed. One lysate was added directly to 100µl of MagneGST™ Particles without removal of cell debris (**Panel A**). The other lysate was cleared by centrifugation at 12,000 × *g* for 40 minutes before adding 100µl of MagneGST™ Particles (**Panel B**). Both samples were then processed according to the MagneGST™ System purification protocol shown in Figure 1.

Purification of Different Sizes of GST-Fusion Proteins

There are several reports indicating that the affinity of GST-fusion proteins for glutathione resin decreases with increasing protein size. Furthermore, a relationship between protein molecular weight and glutathione agarose binding capacity has been shown (7,8). With this in mind we examined purification of proteins ranging from 26kDa to 87kDa. All proteins tested were recovered with high yield and purity (Figure 4). Thus MagneGST™ resin can be used to purify a variety of different protein sizes.

Scaled-up Purification of GST-Tagged Proteins

The characterization of GST-tagged proteins typically involves the screening of many clones in small-scale cultures (e.g., 1ml cultures) to identify the clone that exhibits optimal expression. Further optimization on a small scale is sometimes required to find the best host strain or growth conditions. However, since more protein is sometimes required for analysis, we tested purification of proteins from larger volumes— 5, 10 and 50ml bacterial cultures— using the same basic protocol and scaling the resin and buffer volumes proportionally.

In our experience the MagneGST™ System can be used for purification of small or large proteins from larger volumes of bacterial culture using a proportionally scaled version of the method that is described in Figure 1.

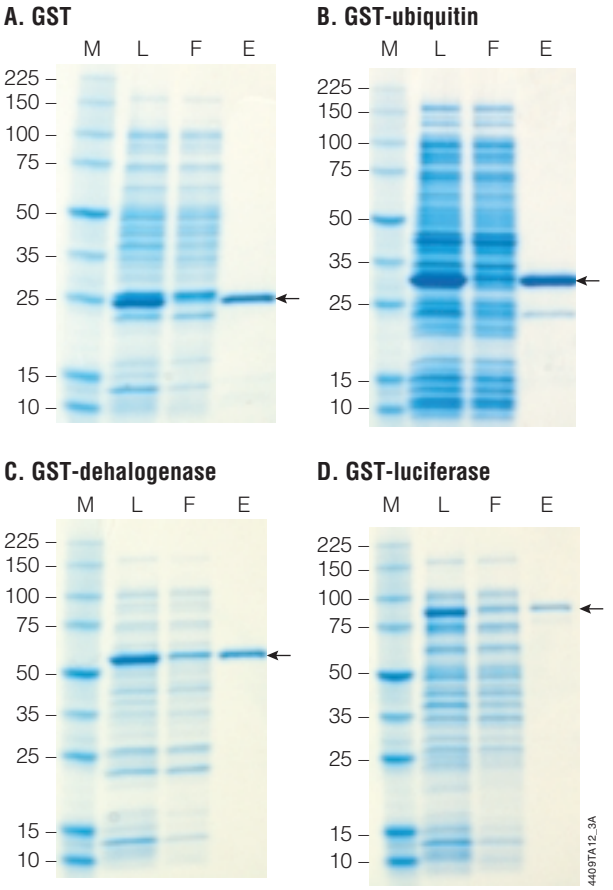

Figure 4. Purification of GST-fusion proteins of different sizes. The MagneGST™ Protein Purification System was used to purify four different sized proteins. One mililiter of crude bacterial lysate was mixed with 100µl of MagneGST™ Particles and purified according to the protocol shown in Figure 1. Samples were analyzed by Western blot. Arrows on the right of each panel indicate the purified protein. **Panel A.** GST. **Panel B.** GST-ubiquitin. **Panel C.** GST-dehalogenase. **Panel D.** GST-luciferase. M: Broad Range Protein Molecular Weight Markers (Cat.# V8491). L: lysate; F: flowthrough; E: eluate.

Purification of GST-Tagged Proteins Expressed In Vitro

Increasingly researchers are using in vitro expression systems to synthesize proteins of interest. It is challenging to purify in vitro-expressed proteins because they are present in very small amounts, typically 150–300ng. A scaled-down version of the MagneGST™ System protocol was employed to successfully purify GST-dehalogenase from the *E. coli* S30 Extract System for Circular DNA[b,c] (Cat.# L1130). GST fusion proteins were detected by Western blot using an anti-GST antibody (data not shown), demonstrating that GST-fusion protein can be recovered from in vitro protein expression systems, even when the expression level is very low.

Automated Purification of GST-Tagged Proteins

As the field of proteomics advances, products that can be used with high-throughput applications and that are amenable with robotic platforms are needed. In addition to being well-suited to manual processing of a small number of samples, the MagneGST™ System is designed to process many samples simultaneously on robotic platforms. We have developed methods that can purify up to 96 GST-fusion proteins, using the Beckman Coulter Biomek® 2000 and Biomek® FX platforms, in as little as 2.5 hours and 1.25 hours, respectively (9). Bacterial cultures grown in a 96-well deep-well plate are centrifuged, the media discarded and the plate placed on the robotic platform for processing.

To test well-to-well variability, a 100ml BL21(DE3) *E. coli* culture expressing a GST-tagged dehalogenase (62kDa) was grown and dispensed in a plate as 96 × 1ml aliquots. (For the purpose of this experiment, the culture was not grown directly in the 96-well plate to eliminate potential well-to-well culture variability.) Protein purification was then carried out on the Beckman Coulter Biomek® 2000. Figure 5 demonstrates consistent yields across the plate. Similar results were obtained with the Beckman Coulter Biomek® FX (data not shown).

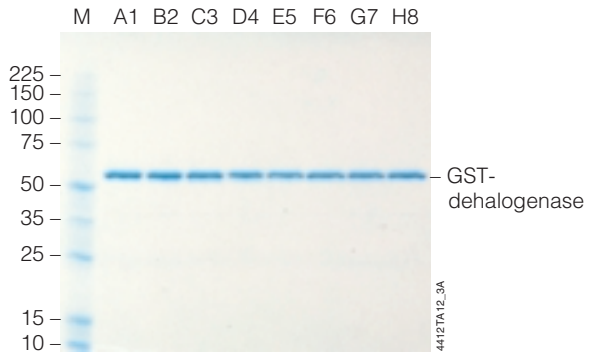

Figure 5. Automated protein purification of GST-dehalogenase fusion protein using MagneGST™ purification resin. GST-dehalogenase was purified from 1ml bacterial cultures in a 96-well format on the Beckman Biomek® 2000, using MagneGST™ Particles and the procedure outlined in Figure 1. Random samples were selected, and a portion of the eluates (2.5%) were analyzed by SDS-PAGE. This data demonstrates consistent yields across the plate. The designations A1–H8 refer to location of well on the plate. Lane M: Broad Range Protein Molecular Weight Markers (Cat.# V8491).

Conclusion

Data presented here show that the MagneGST™ Particles allow efficient purification of GST-fusion proteins of different molecular weights, with good yield and low or no background. The capacity of the MagneGST™ Particles, 7mg of GST-protein per mililiter of settled particles, is equivalent to that of nonmagnetic purification resins. MagneGST™ Particle-based purification is a complete system with all buffers required for protein purification, including cell lysis buffer. The protocol was optimized for purification of proteins directly from crude lysate, without the need for cell debris removal after lysis. The protocol is user friendly and allows purification of GST-fusion proteins in less than 2 hours. In addition to manual purification we also developed protocols for automated purification on the Beckman Coulter Biomek® 2000 and Biomek® FX platforms. The main application for the MagneGST™ System is small-scale purification (1ml cultures); however, it can be successfully used with larger culture volumes (50ml) and for purification of very small amounts of proteins expressed in an in vitro system.

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Protocol

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

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Ordering Information

Product	Size	Cat.#
MagneGST™ Protein Purification System[a]	40 reactions	V8600
	200 reactions	V8603
MagneGST™ Glutathione Particles[a]	4ml	V8611
	20ml	V8612

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