Choosing the Right Protein Purification System

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Abstract

Choosing the best system for purifying fusion-tagged proteins can be overwhelming. Whether you need a large-scale purification for crystallography, a small-scale purification to verify fusion protein production or plan to use the isolated protein to test protein:protein interactions, there is a suitable product for your needs. Promega offers multiple protein purification systems for both large- or small-scale isolation that can be used in a column, batch, automated or magnetic format. The three purification products highlighted here are MagneGST™ Protein Purification System, MagneHis™ Protein Purification System and HisLink™ Protein Purification Resin.

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Choosing a System

To determine which system best meet your needs, consider the following questions:

Which fusion tag is being expressed?

For what applications will the isolated protein be used?

What scale isolation is required?

What binding capacity do you need?

Is the system amenable to automation?

Q Which fusion tag is being expressed?

Promega protein purification systems use one of two fusion tags: Glutathione-S-transferase (GST) or polyhistidine. For isolation of GST fusion proteins, the MagneGST™ Protein Purification System[a] uses glutathione immobilized to paramagnetic particles. The MagneGST™ Particles[a] are composed of cross-linked agarose encapsulating iron oxide and bind GST-tagged proteins directly from a crude lysate. The particles are captured with a magnetic stand, washed, and the GST-fusion target protein is recovered by elution with 50mM glutathione.

There are two systems to choose from for purification of polyhistidine-tagged proteins: MagneHis™ Protein Purification System[b,c] with paramagnetic, precharged nickel particles and HisLink™ Protein Purification Resin[c] with a nonmagnetic resin of nickel-silica. As with the MagneGST™ Protein Purification System, the MagneHis™ Protein Purification System can isolate expressed proteins with a polyhistidine tag from a crude lysate. The protein is bound and captured on the MagneHis™ Ni-Particles[b], impurities are washed away and the His-tagged protein is eluted using a HEPES/imidazole buffer for nondenaturing conditions and a HEPES/imidazole/guanidine-HCl buffer for denaturing conditions. The HisLink™ Protein Purification Resin offers several options: A crude lysate can be used in batch binding, but a cleared lysate is needed prior to gravity or vacuum column purification as well as FPLC or automated purification.

Q For what applications will the purified proteins be used?

When determining what protein isolation system to use, one of the primary considerations is the intended use of the purified protein. Will the protein need to be folded correctly? Correct folding is less critical for proteins used for production of antibodies, while proteins used in activity assays should fold correctly. Isolation of the purified protein under nondenaturing or denaturing conditions will also depend on whether or not the tagged protein forms occlusion bodies when produced in *E. coli*. In some cases, the GST tag can overcome the insolubility; otherwise, polyhistidine-tagged proteins can be purified using denaturing conditions. Structure is important for purification using MagneGST™ Particles. That is, the correct folding of the 26kDa GST tag is needed to bind to the glutathione particles. The His-tag, which is much smaller at only 0.8kDa, is minimally immunogenic and needs little structure, relying on the ability to bind charged nickel ions for purification rather than a structure-based interaction like GST.

The MagneGST™ Protein Purification System can isolate proteins expressed with the TNT® Coupled Reticulocyte Lysate System[d,e,f,g]. Hemoglobin does not bind to the MagneGST™ Particles, minimizing nonspecific background binding. Conversely, the MagneHis™ Ni-particles co-purify the hemoglobin present in the rabbit reticulocyte lysate. This makes the MagneGST™ Particles particularly amenable to GST pull-down procedures to test protein:protein interactions.

What scale isolation is required?

Small-scale purification can be performed with the MagneGST™ and MagneHis™ Protein Purification Systems using 1–50ml of starting culture. Purifications can be performed in batch format without prior clearing of the lysate. Large-scale purification is better accomplished using the nickel-silica resin of the HisLink™ Protein Purification Resin. This resin is scalable for larger starting volumes from 50ml to ≥1L of *E. coli* culture with or without clearing of the lysate. The purification can be performed in either batch or column format, using either gravity or vacuum processing. The HisLink™ Resin is compatible with FPLC or automated purification methods, as the silica resin is noncompressible. In addition, the silica resin is modified with the same proprietary chelator used for the MagneHis™ Ni-Particles.

What binding capacity do you need?

Since the resin or particles used to bind the fusion-tagged proteins are different for each system, there are different binding capacities for each. The MagneHis™ Protein Purification System has a binding capacity of 1mg protein per milliliter of MagneHis™ Particles. The MagneGST™ Protein Purification System has a capacity of 5–10mg GST protein per 1ml of packed MagneGST™ Particles. The HisLink™ Resin is able to bind >15mg of a 20kDa protein per 1ml of packed resin. For all protein purification systems, the size of the protein will impact the binding capacity and recovery of the fusion protein of interest.

Is the system amenable to automation?

Both the MagneGST™ Protein Purification System and MagneHis™ Protein Purification System can be automated using liquid handlers. There are automated methods written for the Beckman Coulter Biomek® 2000 and FX using the MagneGST™ Particles.

The MagneHis™ System can be used with both the Beckman Coulter Biomek® 2000 and FX instruments. Using the FastBreak™ Cell Lysis Reagent[b,c,h] and the MagneHis™ Protein Purification System, automated processing is even easier, as the lysis reagent can be added to the cells with media and placed directly on the robotic platform after a short incubation. Automated methods are available online at: www.promega.com/automethods/

Conclusion

The protein purification systems from Promega accommodate large- and small-scale isolation, allow choice of fusion tags, can be automated and can be used without lysate clearing. The MagneHis™ and HisLink™ Systems allow isolation of the tagged protein under denaturing conditions. The flexibility and ease-of-use of these various systems gives you options to choose the protein purification method best suited for your needs.

Protocols

- *MagneHis™ Protein Purification System Technical Manual* #TM060, Promega Corporation. (www.promega.com/tbs/tm060/tm060.html)
- *HisLink™ Protein Purification Resin Technical Bulletin* #TB327, Promega Corporation. (www.promega.com/tbs/tb327/tb327.html)
- *MagneGST™ Protein Purification System Technical Manual* #TM240, Promega Corporation. (www.promega.com/tbs/tm240/tm240.html)

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[b]Patent Pending.

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[d]U.S. Pat. Nos. 5,324,637 and 5,492,817, Australian Pat. No. 660329 and other patents.

[e]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents and patents pending.

[f]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

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