TECHNICAL MANUAL

MagneGST™ Protein Purification System

Instructions for Use of Products
V8600, V8603, V8611 and V8612

Revised 8/14
TM240

MagneGST™ Protein Purification System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The MagneGST™ Protein Purification System[a,b] provides a simple, rapid and reliable method to purify glutathione-S-transferase (GST) fusion proteins (Figure 1). The use of nonmagnetic GST purification matrices for small-scale batch protein purifications can be time-consuming and difficult. The use of paramagnetic particles eliminates several centrifugation steps and the need for multiple tubes. It also minimizes the loss of sample material. Glutathione immobilized on paramagnetic particles (MagneGST™ Glutathione Particles) is used to isolate GST-fusion proteins directly from a crude cell lysate using a manual or automated procedure. Using the manual protocol, GST-fusion proteins can be purified on a small scale from 1ml of culture or on a large scale from up to 50ml of culture. Although the MagneGST™ System is designed for manual applications, samples also can be processed using robotic platforms for high-throughput applications.

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1. Description (continued)

Bacterial cells containing a GST-fusion protein are lysed using the provided MagneGST™ Cell Lysis Reagent or with an alternative lysis method, and the MagneGST™ particles are added directly to the crude lysate. GST-fusion proteins bind to the MagneGST™ particles. Unbound proteins are washed away, and the GST-fusion target protein is recovered by elution with 50mM glutathione (Figure 1). Examples of proteins purified by this method are shown in Figure 2. Properties of the MagneGST™ particles are given in Table 1.

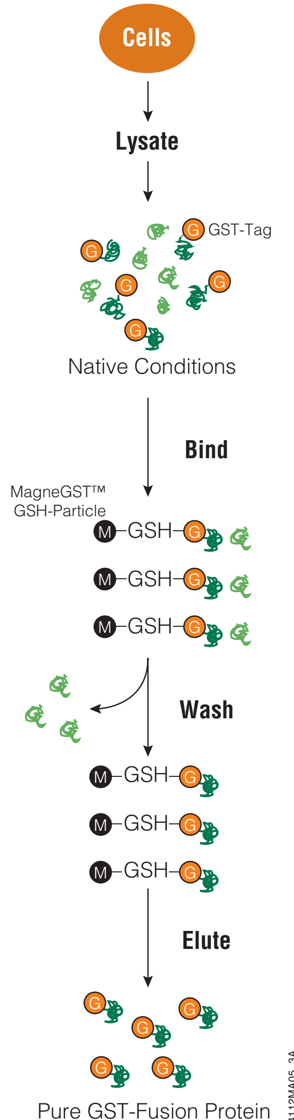

Figure 1. Schematic diagram of the MagneGST™ Protein Purification System protocol.

Table 1. Characteristics of MagneGST™ Particles.

Composition:	Reduced glutathione (GSH) is covalently attached to the surface of the particles via a sulfide linkage using a 12-atom linker. The particles are cross-linked agarose encapsulating iron oxide
Particle Size:	Approximately 1–10 microns.
Concentration:	The MagneGST™ Glutathione Particles are supplied as a 25% slurry in 20% ethanol.
Binding Capacity:	One milliliter of settled particles will bind 5–10mg of GST protein. **Note:** 1ml of settled particles corresponds to 4ml of the 25% slurry supplied.

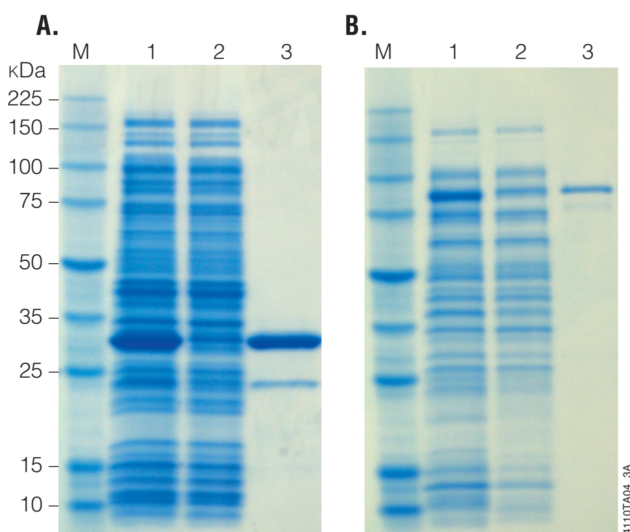

Figure 2. Purification of GST-fusion proteins using the MagneGST™ Protein Purification System. Panel A. Purification of a GST-ubiquitin fusion protein. Lane M, protein molecular weight marker; lane 1, crude bacterial cell lysate containing the GST-ubiquitin fusion protein; lane 2, flowthrough fraction; lane 3, GST-ubiquitin protein eluted from the MagneGST™ particles. **Panel B.** Purification of a GST-luciferase fusion protein. Lane M, protein molecular weight marker; lane 1, crude bacterial cell lysate containing the GST-luciferase fusion protein; lane 2, flowthrough fraction; lane 3, GST-luciferase protein eluted from the MagneGST™ particles.

1. Description (continued)

Benefits of the MagneGST™ Protein Purification System include:

- **Simple:** Allows one-step purification of multiple samples with easy handling.
- **Quick:** No requirement for high-speed centrifugation to clarify the lysate once cells are lysed.
- **Scalable:** Volumes can be adjusted according to the amount of starting material, from 1ml to 50ml of cell culture.
- **Efficient:** High yields with little or no nonspecific background.
- **Versatile:** Purification can be performed manually or using an automated platform.
- **Magnetic-Based:** Minimizes sample loss that occurs in batch purification with sepharose-based resins.
- **Convenient:** Complete system includes all necessary components, including a unique MagneGST™ Cell Lysis Reagent and RQ1 RNase-Free DNase.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
MagneGST™ Protein Purification System	**40 purifications**	**V8600**

Each system contains sufficient reagents for 40 manual purifications from 1ml of bacterial cell culture. Includes:

- 80ml MagneGST™ Binding/Wash Buffer
- 2ml Glutathione, 0.5M Solution
- 12ml MagneGST™ Cell Lysis Reagent
- 2ml Tris Buffer, 0.5M (pH 8.1)
- 4ml MagneGST™ Glutathione Particles
- 1,000 units RQ1 RNase-Free DNase

PRODUCT	SIZE	CAT.#
MagneGST™ Protein Purification System	**200 purifications**	**V8603**

Each system contains sufficient reagents for 200 manual purifications from 1ml of bacterial cell culture. Includes:

- 400ml MagneGST™ Binding/Wash Buffer
- 10ml Glutathione, 0.5M Solution
- 60ml MagneGST™ Cell Lysis Reagent
- 10ml Tris Buffer, 0.5M (pH 8.1)
- 2 × 10ml MagneGST™ Glutathione Particles
- 1,000 units RQ1 RNase-Free DNase

PRODUCT	SIZE	CAT.#
MagneGST™ Glutathione Particles	**4ml**	**V8611**

- 2 × 10ml V8612

Storage Conditions: Store the Glutathione, 0.5M Solution, at –20°C for up to 3 months or at –70°C for long-term storage. When using the system for the first time, thaw the Glutathione and dispense into small aliquots of 100–500µl. Do not freeze and thaw the Glutathione more than five times. Store the RQ1 RNase-Free DNase at –20°C. Store all other components at 4°C. The Tris Buffer, 0.5M (pH 8.1), MagneGST™ Binding/Wash Buffer and MagneGST™ Cell Lysis Reagent also can be stored at room temperature. **Do not freeze the MagneGST™ Glutathione Particles.**

3. Before You Begin

Materials to be Supplied by the User

- 37°C incubator for growing bacterial culture
- 25°C incubator for inducing protein expression (optional)
- centrifuge
- magnetic separation stand (see note below)
- NANOpure® or double-distilled water

Note: This protocol requires a magnetic separation stand. The MagneSphere® Technology Magnetic Separation Stand (twelve-position) (Cat.# Z5342) can be used to hold one to twelve 1.5ml microcentrifuge tubes. The MagneSphere® Technology Magnetic Separation Stand (twelve-position) (Cat.# Z5343) can be used to hold one to twelve 75mm tubes. The MagneSphere® Technology Magnetic Separation Stand (two-position) (Cat.# Z5332) can be used to hold two 1.5ml microcentrifuge tubes. The MagneSphere® Technology Magnetic Separation Stand (two-position) (Cat.# Z5333) can be used to hold two 75mm tubes. The MagneSil® Magnetic Separation Unit (Cat.# A2231) can be used to hold a single 15ml or 50ml conical tube.

3.A. Preparation of Cells

Bacterial cultures can be grown in tubes, flasks or 96-well deep-well plates (Marsh Bio Products Cat.# AB-0932). Grow the culture containing the appropriate GST-fusion protein to an $O.D._{600}$ between 0.4 and 0.6, then induce protein expression. For IPTG induction, add IPTG to a final concentration of 1mM and incubate at 37°C for 3 hours or at 25°C overnight. Depending upon the expression system, other methods of induction and growth conditions can be used. Induction conditions often require optimization. We recommend that cell cultures have a final $O.D._{600}$ ≤6 (see Section 5.B). For 1ml bacterial cultures, pellet cells by centrifugation at 16,000 × *g* for 3 minutes and carefully remove the supernatant. For culture volumes larger than 1ml, pellet the cells by centrifugation at 3,000 × *g* for 20 minutes. Carefully remove the supernatant. The cell pellet can be frozen at –20°C or at –70°C for long-term storage, or the cells can be lysed immediately.

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3.B. Preparation of Elution Buffer

1. When using the system for the first time, thaw the Glutathione and dispense into small aliquots of 100–500µl. Store the aliquots at –20°C for up to 3 months or at –70°C for long-term storage. Do not freeze and thaw the Glutathione more than five times.
2. Determine the volume of elution buffer required for elution. Each purification requires 200µl of elution buffer. For each 1ml of elution buffer needed, mix 100µl of 0.5M Glutathione and 100µl of Tris Buffer, 0.5M (pH 8.1) with 800µl of NANOpure® or double-distilled water. The final concentration will be 50mM Glutathione and 50mM Tris-HCl (pH 8.1).

   Note: The elution buffer can be stored at –20°C for up to 1 month.

This recommended Glutathione concentration will elute most GST-fusion proteins. See Section 5.A for alternative elution conditions.

4. MagneGST™ Protein Purification Protocols

Materials to be Supplied by the User

- 1.5ml microcentrifuge tubes for small-scale protein purifications or 15ml or 50ml conical tubes for large-scale protein purifications
- magnetic separation stand (See note in Section 3.)
- shaker or rotating platform
- centrifuge

Note: Use tubes that can accommodate the volume of the MagneGST™ particles and MagneGST™ Binding/Wash Buffer required for the purification. The tubes must be compatible with a magnetic separation stand. It may be necessary to use multiple tubes.

4.A. Small-Scale Purification Using the MagneGST™ Cell Lysis Reagent (1ml cultures)

Small-scale purification of GST-fusion proteins using the MagneGST™ Protein Purification System is useful for screening multiple clones for expression, optimizing expression conditions (temperature, medium, host strain, etc.) and the initial screening of mutant clones.

Cell Lysis

1. Prepare cell pellets from 1ml of bacterial culture as described in Section 3.A. Remove all growth medium.

   Recommended: Freeze the cell pellets at –20°C (15–20 minutes is usually sufficient) or on dry ice for 5–10 minutes. Freezing cell pellets will increase efficiency of cell lysis of certain strains, such as the BL21 series of strains, or when cultures are grown to a high density. For cultures of $O.D._{600}$ >2, more lysis buffer will be required.
2. Add 200µl of MagneGST™ Cell Lysis Reagent to each fresh or frozen cell pellet. Resuspend the cell pellet at room temperature (20–25°C) by pipetting or gentle mixing.

   Note: The MagneGST™ Cell Lysis Reagent was designed for use with the MagneGST™ System. Other commercially available lysis reagents may not work properly with this system. For cultures of $O.D._{600}$ >2, add 100µl of the MagneGST™ Cell Lysis Reagent per 1 $O.D._{600}$ (i.e., for a culture $O.D._{600}$ = 3.0, use 300µl MagneGST™ Cell Lysis Reagent).

3. Add 2µl of RQ1 RNase-Free DNase.

   Note: Addition of DNase reduces viscosity and can increase the purity of GST-fusion proteins. Up to 5µl of RQ1 RNase-Free DNase can be added to reduce viscosity, if needed. The DNase can be omitted, if desired.

4. Incubate the cell suspension at room temperature for 20–30 minutes on a rotating platform or shaker.

We have found that the MagneGST™ Cell Lysis Reagent efficiently lyses *E. coli* strains BL21(DE3)pLysS and JM109. Maximal lysis in some strains may require addition of lysozyme or a freeze-thaw cycle. Refer to Section 5.B for details.

Particle Equilibration

1. Thoroughly resuspend the MagneGST™ particles by inverting the bottle to obtain a uniform suspension.
2. Pipet 100µl of MagneGST™ particles into a 1.5ml tube.
3. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet. Magnetic capture of the particles will typically occur within a few seconds.
4. Carefully remove and discard the supernatant.
5. Remove the tube from the magnetic stand. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles, and resuspend by pipetting or inverting.
6. Repeat Particle Equilibration Steps 3–5 twice for a total of 3 washes.

Binding

1. After the final wash, gently resuspend the particles in 100µl of MagneGST™ Binding/Wash Buffer.

   Note: After being washed and resuspended in the appropriate volume of MagneGST™ Binding/Wash Buffer, the MagneGST™ particles can be stored at 4°C overnight. Do not allow the particles to dry.

2. Add 200µl of cell lysate, prepared as described above, to the particles. Mix gently by pipetting or inverting. If the combined volume of cell lysate and MagneGST™ particles is less than 300µl, add additional MagneGST™ Binding/Wash Buffer so that the final volume is 300µl.
3. Incubate with gentle mixing on a rotating platform or shaker for 30 minutes at 4°C or room temperature.

   Note: Do not allow particles to settle for more than a few minutes, as this will reduce binding efficiency.

Washing

1. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
2. Carefully remove the supernatant. Save the supernatant (flowthrough) for SDS-PAGE analysis, if desired.
3. Remove the tube from the magnetic stand. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles, and mix gently by pipetting or inverting. Incubate at room temperature or 4°C for 5 minutes. Occasionally mix by inverting the tube.
4. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
5. Carefully remove the supernatant. Save the supernatant if analysis of wash solution is desired.

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4.A. Small-Scale Purification Using the MagneGST™ Cell Lysis Reagent (1ml cultures) (continued)

6. Remove the tube from the magnetic stand. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles, and mix gently by pipetting or inverting. An incubation is not necessary at this step.
7. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
8. Carefully remove the supernatant. Save the supernatant if analysis of wash solution is desired.
9. Repeat Washing Steps 6–8 once for a total of 3 washes.

Elution

1. After the final wash, add 200µl of the elution buffer prepared in Section 3.B.

   Note: Smaller volumes of elution buffer can be used. See Section 5.A.
2. Incubate at room temperature or 4°C for 15 minutes with gentle mixing.

   Note: Do not allow particles to settle for more than a few minutes, since inefficient mixing will decrease elution efficiency.
3. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
4. Carefully remove the supernatant, and transfer it to a clean tube. The supernatant contains the eluted GST-fusion protein.
5. If a second elution is desired, repeat Elution Steps 1–4.

4.B. Small-Scale Protein Purification From Cells Lysed by Sonication or Other Methods

Cells may be lysed using sonication, repeated freeze-thaw cycles, a French press, lysozyme or other methods. In this protocol, cell lysates are prepared so that the volume of the cell lysate is 1/20 that of the original culture volume. This protocol is adjusted for 50µl of cleared cell lysate, which corresponds to 1ml of bacterial culture. If cell lysates are more concentrated or other lysate volumes are used, adjust the amount of particles and all buffers accordingly.

Cell Lysis

1. Prepare cell pellets from 1ml of bacterial culture as described in Section 3.A.
2. Resuspend each fresh or frozen cell pellet in 50µl of MagneGST™ Binding/Wash Buffer or buffer of choice by pipetting or inverting at room temperature (20–25°C) or 4°C.

   Note: Cell pellets also can be resuspended with the MagneGST™ Cell Lysis Reagent prior to lysis with other methods.
3. Disrupt resuspended cells using sonication, repeated freeze-thaw cycles, a French press, lysozyme or other means of cell lysis.

   Note: If using sonication, sonicate cells on ice in short bursts. Excessive sonication may lead to protein denaturation and copurification of other host proteins with the GST-fusion protein.
4. Centrifuge lysed cells at 12,000 × *g* for 40 minutes at 4°C. Transfer the supernatant to a fresh tube. Reserve a fraction of supernatant and pellet for SDS-PAGE analysis to determine solubility of the protein.

Particle Equilibration

1. Thoroughly resuspend the MagneGST™ particles by inverting the bottle to obtain a uniform suspension.
2. Pipet 100µl of MagneGST™ particles into a 1.5ml tube.
3. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet. Magnetic capture of the particles will typically occur within a few seconds.
4. Carefully remove and discard the supernatant.
5. Remove the tube from the magnetic stand. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles, and resuspend by pipetting or gentle mixing.
6. Repeat Particle Equilibration Steps 3–5 twice for a total of 3 washes.

Binding

1. After the final wash, gently resuspend the particles in 250µl of MagneGST™ Binding/Wash Buffer.

   Note: After being washed and resuspended in the appropriate volume of MagneGST™ Binding/Wash Buffer, the MagneGST™ particles can be stored at 4°C overnight. Do not allow the particles to dry.
2. Add 50µl of cleared cell lysate to the particles. If the combined volume of cell lysate and MagneGST™ Particles is less than 300µl, add additional MagneGST™ Binding/Wash Buffer so that the final volume is 300µl.
3. Incubate with gentle mixing on a rotating platform or shaker for 30 minutes at 4°C or room temperature.

   Note: Do not allow particles to settle for more than a few minutes, as this will reduce binding efficiency.

Washing

1. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
2. Carefully remove the supernatant. Save the supernatant (flowthrough) for SDS-PAGE analysis, if desired.
3. Remove the tube from the magnetic stand. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles, and mix gently by pipetting or inverting. Incubate at room temperature for 5 minutes. Occasionally mix by tapping or inverting the tube.
4. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
5. Carefully remove the supernatant. Save the supernatant if analysis of wash solution is desired.
6. Remove the tube from the magnetic stand. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles, and mix gently by pipetting or inverting.
7. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
8. Carefully remove the supernatant. Save the supernatant if analysis of wash solution is desired.
9. Repeat Washing Steps 6–8 once for a total of 3 washes.

4.B. Small-Scale Protein Purification From Cells Lysed by Sonication or Other Methods (continued)

Elution

1. After the final wash, add 200µl of elution buffer.

   Note: Smaller volumes of elution buffer can be used. See Section 5.A.

2. Incubate with gentle mixing on a rotating platform or shaker at room temperature or 4°C for 15 minutes.

   Note: Do not allow particles to settle for more than a few minutes, since inefficient mixing will decrease elution efficiency.

3. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.

4. Carefully remove the supernatant, and transfer it to a clean tube. The supernatant contains the eluted GST-fusion protein.

5. If a second elution is desired, repeat Elution Steps 1–4.

4.C. Large-Scale Purification (1–50ml Cultures or an Equivalent Amount of Cleared Lysate)

Cells may be lysed using the MagneGST™ Cell Lysis Reagent or by other methods (see Section 4.B). Cell lysates prepared with the MagneGST™ Cell Lysis Reagent do not need to be cleared by centrifugation.

If an alternative method of lysis is employed, add to the cell pellet a volume of MagneGST™ Binding/Wash Buffer that is equal to 1/20 of the original culture volume. For example, resuspend the cell pellet from a 50ml bacterial culture in 2.5ml of MagneGST™ Binding/Wash Buffer. Other volumes of MagneGST™ Binding/Wash Buffer can be used to resuspend the cell pellet, resulting in more or less concentrated cell lysates. If cell lysates are more concentrated or other lysate volumes are used, adjust the amount of particles and all buffers accordingly. After cell lysis using lysis methods other than the MagneGST™ Cell Lysis Reagent, we recommend clearing the lysate by centrifugation at 12,000 × *g* for 40 minutes at 4°C. Use the cleared lysate (supernatant) for protein purification.

This protocol is written for protein purification from 10ml of original culture volume using the MagneGST™ Cell Lysis Reagent to prepare the cell lysate. For culture volumes other than 10ml, linearly scale up or down the volumes of MagneGST™ particles (see Table 2) and all buffers for binding, washing and eluting.

Cell Lysis Using the MagneGST™ Cell Lysis Reagent

1. Prepare cell pellets from 1–50ml of bacterial culture as described in Section 3.A.

   Recommended: Freeze the cell pellets at –20°C (15–20 minutes is usually sufficient although large cell pellets may require more time to freeze completely) or on dry ice for 5–10 minutes. Freezing cell pellets will increase efficiency of cell lysis of certain strains, such as the BL21 series of strains, or cultures that are grown to a high density.

2. Resuspend the fresh or frozen cell pellets in MagneGST™ Cell Lysis Reagent. Use 2ml of MagneGST™ Cell Lysis Reagent per 10ml of original culture volume (see Table 2). Mix by pipetting or gentle mixing. For cultures at 6.0 $O.D._{600}$, use 6ml of MagneGST™ Cell Lysis Reagent per 10ml of original culture volume.

Table 2. Recommended Volumes of MagneGST™ Cell Lysis Reagent for Cell Resuspension.

Original Culture Volume	MagneGST™ Cell Lysis Reagent Volume ($O.D._{600}$ = 2.0)	MagneGST™ Cell Lysis Reagent Volume ($O.D._{600}$ = 6.0)
1ml	200µl	600µl
5ml	1ml	3ml
10ml	2ml	6ml
50ml	10ml	30ml

3. Add 20µl of RQ1 RNase-Free DNase per 10ml of original culture volume.

   Note: Addition of DNase reduces viscosity and can increase the purity of GST-fusion proteins. Up to 50µl of RQ1 RNase-Free DNase can be added to reduce viscosity, if needed. The DNase can be omitted, if desired, but addition of DNase is highly recommended for higher $O.D._{600}$ cultures.

4. Incubate the cell suspension at room temperature (20–25°C) for 30 minutes on a rotating platform or shaker.

   Optional: After the 30-minute incubation, the insoluble cell debris may be removed by centrifugation at 16,000 × *g* for 40 minutes at 4°C or room temperature (20–25°C). This step may increase yield and purity of some GST-fusion proteins. We recommend this step when cells are lysed by methods other than the use of the MagneGST™ Cell Lysis Reagent.

Particle Equilibration

1. Thoroughly resuspend the MagneGST™ particles by inverting the bottle to obtain a uniform suspension.
2. For every 10ml of original culture volume, pipet 1ml of MagneGST™ particles into a conical tube (see Table 3).

Table 3. Recommended Volume of MagneGST™ Particles for Protein Purification.

Original Culture Volume	Volume of MagneGST™ Particles
1ml	100µl
5ml	500µl
10ml	1ml
50ml	5ml

   Note: The volume of particles will depend on the level of GST-fusion protein expression. In some cases, a smaller volume of MagneGST™ particles may be sufficient (e.g., 2.5ml of MagneGST™ particles may be sufficient to bind the protein in a 50ml culture).

3. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet. Magnetic capture of the particles will typically occur within a few seconds.
4. Carefully remove and discard the supernatant.

4.C. Large-Scale Purification (1–50ml Cultures or an Equivalent Amount of Cleared Lysate) (continued)

5. For every 10ml of original culture volume, add 2.5ml of MagneGST™ Binding/Wash Buffer to the particles. Resuspend by pipetting or inverting.
6. Repeat Particle Equilibration Steps 3–5 twice for a total of 3 washes.

Binding

1. After the final wash, gently resuspend the particles in 1ml of MagneGST™ Binding/Wash Buffer per 10ml of original culture volume.
2. Add 2ml of cell lysate to the particles. If more concentrated lysates are used, the combined volume of cell lysate and MagneGST™ particles may be less than 3ml. Add additional MagneGST™ Binding/Wash Buffer so that the final volume is 3ml.
3. Incubate with gentle mixing on a rotating platform or shaker for 30 minutes at 4°C or room temperature.

   Note: Do not allow particles to settle for more than a few minutes, as this will reduce binding efficiency.

Washing

1. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
2. Carefully remove the supernatant. Save the supernatant (flowthrough) for SDS-PAGE analysis, if desired.
3. Remove the tube from the magnetic stand. Add to the particles 2.5ml of MagneGST™ Binding/Wash Buffer per 10ml of original culture volume, and mix by pipetting or inverting. Incubate for 5 minutes at room temperature or 4°C. Occasionally mix by inverting the tube.
4. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
5. Carefully remove the supernatant. Save the supernatant if analysis of wash solution is desired.
6. Remove the tube from the magnetic stand. Add to the particles 2.5ml of MagneGST™ Binding/Wash Buffer per 10ml of original culture volume, and mix gently by pipetting or inverting.
7. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
8. Carefully remove the supernatant. Save the supernatant if analysis of wash solution is desired.
9. Repeat Washing Steps 6–8 once for a total of 3 washes.

Elution

1. After the final wash, add 2ml of elution buffer per 10ml of original culture volume.

   Note: Smaller volumes of elution buffer can be used. See Section 5.A.
2. Incubate with gentle mixing on a rotating platform or shaker for 15 minutes at 4°C or room temperature.

   Note: Do not allow particles to settle for more than a few minutes, as this will reduce elution efficiency.
3. Place the tube in the magnetic stand, and allow the MagneGST™ particles to be captured by the magnet.
4. Carefully remove the supernatant, and transfer it to a clean tube. The supernatant contains the eluted GST-fusion protein.
5. If a second elution is desired, repeat Elution Steps 1–4.

5. General Considerations

5.A. Alternative Elution Conditions

The composition of the recommended elution buffer was selected to effectively elute most GST-fusion proteins. However, a notable amount of protein may remain bound to the particles, particularly when purifying large proteins. In these cases, alternative elution conditions may be required for maximum protein yield. The composition of the elution buffer also can be altered to suit the requirements of specific downstream applications (e.g., mass spectrometry analysis or enzymatic assays). Adjusting the glutathione, Triton® X-100 and NaCl concentrations in the elution buffer will have the greatest effect on elution efficiency. Increasing any one of these components will increase elution efficiency of proteins that have proven difficult to elute. Addition of one or more of these reagents at the highest concentrations listed below is compatible with the MagneGST™ particles.

Glutathione

Glutathione at concentrations as low as 10mM will elute most small (<50kDa) proteins. As the size of the protein increases, more glutathione may be needed to effectively elute the protein. Glutathione must be present to elute the GST-fusion proteins from MagneGST™ particles. Concentrations of up to 100mM glutathione have been found to improve elution efficiency of difficult-to-elute proteins.

Triton® X-100

Addition of up to 1% Triton® X-100 has been shown to be effective in eluting some proteins in the presence of glutathione. Addition of this reagent is compatible with up to 100mM glutathione and 500mM NaCl.

Sodium Chloride

Sodium chloride can be added to the elution buffer to disrupt ionic interactions between the GST-fusion protein and MagneGST™ particles. Addition of up to 500mM NaCl can be helpful in eluting difficult-to-elute proteins.

Elution Volume

To obtain a more concentrated protein sample, the volume of elution buffer can be decreased to one half or one-third of the original recommended volume. However, the total recovery of the protein may be decreased. In some cases, larger elution volumes can increase yield.

5.B. Cell Lysis Considerations

The MagneGST™ Cell Lysis Reagent will lyse fresh or frozen cells under most conditions. However, certain conditions, such as high cell density (O.D. >6) or the use of certain strains (e.g., BL21 strains), may affect lysis efficiency. Furthermore, cells grown in different media may be lysed with different efficiencies. We have found that cells grown to the same cell density in Terrific broth were more efficiently lysed than cells grown in Luria broth. Certain strains, such as BL21(DE3), grown in Luria broth to O.D. >4, may not be completely lysed unless cell pellets are frozen and thawed or additional MagneGST™ Cell Lysis Reagent is added. Use 100µl of MagneGST™ Cell Lysis Reagent per 1 $O.D._{600}$ of 1ml culture volume. Under these conditions, freezing and thawing of the cell pellets may be required for complete lysis prior to protein purification. Freezing the cells at –20°C (15–20 minutes is usually sufficient although large cell pellets may require more time to freeze completely) or on dry ice for 5–10 minutes and then thawing increases the efficiency of cell lysis. In some cases, adding lysozyme to a final concentration of 0.2–1mg/ml also can improve cell lysis. Other lysis methods, such as sonication or use of a French press, may be used. Pelleted cells must be thoroughly resuspended in the MagneGST™ Binding/Wash Buffer or your buffer of choice prior to cell lysis when using sonication or other lysis methods. Cell pellets also can be resuspended with the MagneGST™ Cell Lysis Reagent prior to lysis with other methods.

Efficient lysis often results in viscous samples, especially when using the BL21 (DE3)pLysS strain. We recommend adding DNase (2–5 units/ml of culture) to reduce viscosity; this can increase the purity of proteins in some cases. Addition of protease inhibitors during cell lysis may be desired to reduce proteolysis.

5.C. Other Considerations

1. The MagneGST™ particles are compatible with many common buffer components (see Table 4).

Table 4. Tolerance of MagneGST™ Particles Using Alternative Buffers.

Buffer Component	Concentration
DTT	≤10mM
NaCl	≤0.64M
Tris, HEPES, sodium phosphate, potassium phosphate	≤100mM
Triton® X-100 detergent	≤1.0%
Tween® 20	≤1.0%
Mazu	≤1.0%
cetyltrimethylammonium bromide (CTAB)	≤1.0%
ethanol	20%
protease inhibitor cocktail (Roche Molecular Systems, Inc., Cat.# 1836170)	1X

2. MagneGST™ particles should be stored and handled carefully to avoid contamination. Always use new pipette tips. Do not store MagneGST™ particles adjacent to cell cultures, especially when working with yeast cultures.
3. Purified GST-fusion proteins can be quantitated using standard methods such as Bradford or BCA protein assays. Glutathione in the elution buffer may slightly increase absorbance values obtained in a protein assay. Thus, the reference and experimental samples should be adjusted to the same glutathione concentration.

4. If you intend to repurify a sample that was already purified using the MagneGST™ Protein Purification System, you must first reduce the glutathione concentration in the sample by dilution or dialysis. Dilute the sample with MagneGST™ Binding/Wash Buffer or any other buffer that is compatible with downstream applications. Alternatively, dialyze the sample with several changes of MagneGST™ Binding/Wash Buffer or another buffer that is compatible with downstream applications.
5. We have used the MagneGST™ particles to purify GST-fusion protein generated in vitro using the *E. coli* S30 Extract System for Circular DNA (Cat.# L1020). When eluted protein was analyzed by SDS polyacrylamide gel electrophoresis, no major contaminating proteins were found to copurify with the GST-fusion proteins.

6. Isolation of GST-Fusion Proteins Using Laboratory Automation Workstations

The manual protocol described in Section 4 can be used as a guide to develop protocols for automated workstations. The protocol may require optimization, depending on the instrument used. Promega has an ongoing effort to develop procedures for different automated platforms. Specific instructions for use on several automated platforms are available at: **www.promega.com/automethods/**

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
GST-fusion protein not expressed or expressed at low levels	DNA sequence or orientation was not correct. The protein-coding region must be inserted into the vector in the proper orientation and in the correct reading frame. Check the orientation and reading frame by sequencing. Check for the presence of rare codons.
	Cells were not induced. Use the correct inducing agents at the optimal concentration. Optimize other expression conditions, such as bacterial strain, medium composition, temperature and cell density at time of induction. Longer induction times at lower temperatures (10–16 hours at 25°C) can often increase protein expression over that at shorter induction times at 37°C.
	For unstable expressed proteins, add protease inhibitors during the cell lysis step.
	Reduce the induction period to minimize protein degradation at the time of expression.
	Lower the temperature to stablize the protein during induction.

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7. Troubleshooting (continued)

Symptoms	Causes and Comments
GST-fusion protein not expressed or expressed at low levels (continued)	Protein expression level was below the level of detection by SDS-PAGE. Check protein levels by Western blot. Optimize expression conditions such as bacterial strain, medium composition, temperature and cell density at time of induction.
	Induced cells were not growing. Expressed protein may be toxic to the cells. Decrease protein expression by shortening the time of induction or lowering the amount of inducing agent.
GST-fusion protein not binding to the MagneGST™ particles	To ensure sufficient cell lysis, freeze and thaw the cell pellets once or twice prior to adding the MagneGST™ Cell Lysis Reagent to increase efficiency of cell lysis. Lysis efficiencies of different bacterial strains can vary dramatically. BL21(DE3) cells may be more resistant to lysis with the MagneGST™ Cell Lysis Reagent than other strains.
	Poor cell lysis due to insufficient volume of MagneGST™ Cell Lysis Reagent. Increase the volume of MagneGST™ Cell Lysis Reagent, and increase the time of incubation. Cell disruption is evident by partial clearing of the cell suspension upon addition of the MagneGST™ Cell Lysis Reagent. Lysis also can be confirmed visually under a microscope.
	Add 1/10 volume of 10mg/ml lysozyme in 25mM Tris-HCl (pH 8.0) to the MagneGST™ Cell Lysis Reagent to aid lysis.
	Express the protein in a bacterial strain that is known to lyse more efficiently, such as BL21(DE3)pLysS or JM109.
	If sonication was used to disrupt the cells, sonication may not have been sufficient to release the protein. Sonicate cells for a longer period of time.
	Expressed protein is insoluble. Centrifuge a small volume of the lysed cells at 16,000 × *g* for 40 minutes. Separate the pellet and supernatant, and analyze both fractions for the presence of the GST-fusion protein. If the protein is found in the pellet, optimizing growth conditions may increase solubility. For example, lowering the temperature during induction, altering the concentration of inducing agent, inducing the culture for less time at a higher cell density and increasing aeration during growth may decrease formation of inclusion bodies. The addition of Sarkosyl can solubilize many GST-fusion proteins (1).

Symptoms	Causes and Comments
GST-fusion protein not binding to the MagneGST™ particles (continued)	Excessive sonication caused protein denaturation, which can lead to protein insolubility. Keep cells on ice during sonication. Alternate short cycles of sonication and incubation of cells on ice.
	Avoid using microtip sonicators.
	Protein degradation. Add protease inhibitors to cells or MagneGST™ Cell Lysis Reagent prior to cell lysis.
	Binding of GST-fusion proteins are sensitive to pH. Buffers with a pH >8.0 can interfere with protein binding. If cell lysis was not performed in the supplied MagneGST™ Cell Lysis Reagent or MagneGST™ Binding/Wash Buffer, check the pH of the lysis buffer. Use the MagneGST™ Binding/Washing Buffer to lyse cells by alternative lysis methods (see Section 4.B), or add more MagneGST™ Binding/WashingBuffer to the binding reaction.
	Inefficient protein binding. Add DTT to a final concentration of 5mM prior to cell lysis. This has been shown to increase binding of some GST-fusion proteins (1).
	Temperature was too high during binding steps. Lower temperature to 4°C. This can increase binding efficiency.
	Protein was expressed at high levels. The recommended amount of MagneGST™ particles may not be sufficient to bind all of the protein in the cell lysate. Increase the amount of particles, and scale up the volume of MagneGST™ Binding/Wash and elution buffer proportionally. The binding capacity of the MagneGST™ particles is 5–10mg of GST-fusion protein per 1ml of settled particles (1ml of settled particles equals 4ml of the 25% slurry).
	Excessive washing. Limit the number of washes after the binding reaction.
Fusion protein not efficiently eluted from the particles	Poor elution efficiency due to protein size. Larger proteins are eluted less efficiently. Increase the concentration of glutathione in the elution buffer to 100mM.
	Increase the ionic strength of the elution buffer. Add NaCl to the elution buffer to a final concentration of 100–500mM.
	pH is important for efficient elution. Buffers with pH ≥8.0 elute proteins more efficiently than buffers with pH 7.5. Check the pH of the elution buffer. Increasing the pH of the elution buffer may increase recovery.
	Include 0.1–1% Triton® X-100 in the elution buffer.

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7. Troubleshooting (continued)

Symptoms	Causes and Comments
Fusion protein not efficiently eluted from the particles (continued)	Increase the volume of elution buffer, or perform two elution steps.
Multiple proteins observed on SDS-polyacrylamide gel	Protein degradation. Add protease inhibitors to cells or MagneGST™ Cell Lysis Reagent prior to cell lysis.
	Protein aggregation due to excessive sonication. Use milder sonication conditions. Keep cells on ice during sonication. Alternate short cycles of sonication and incubation of cells on ice. Avoid frothing during sonication. Avoid using microtip sonicators.
	Multiple proteins copurified with GST-fusion protein. Increase the number of wash steps.
	Add NaCl to the MagneGST™ Binding/Wash Buffer up to a final concentration of 0.5M to increase stringency. The optimal NaCl concentration may need to be determined empirically.
	After the 20- to 30-minute incubation in the MagneGST™ Cell Lysis Reagent to lyse the cells, insoluble cell debris may be removed by centrifugation at 16,000 × *g* for 20 minutes at 4°C or room temperature. This step may increase yield and purity of some GST-fusion proteins.

8. Reference

1. Frangioni, J.V. and Neel, B.G. (1993) Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins. *Anal. Biochem.* **210**, 179–87.

9. Appendix

9.A. Optical Density Calculation

The optical density (O.D.) of a bacterial culture measured at 600nm is often used to monitor cell growth. We recommend growing the bacterial culture to an $O.D._{600}$ between 0.4 and 0.6 before inducing protein expression. If necessary, measure the $O.D._{600}$ of a diluted culture and calculate the $O.D._{600}$ of the undiluted culture:

$O.D._{600} = 10 \times O.D._{600}$ of 1ml of a 1:10 dilution of the culture (diluted in medium) measured in a 1cm pathlength cuvette

9.B. Composition of Buffers and Solutions

4X SDS gel-loading buffer

0.24M	Tris-HCl (pH 6.8)
2%	SDS
3mM	bromophenol blue
50.4%	glycerol
0.4M	dithiothreitol

SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should be added from a 1M stock just before the buffer is used.

MagneGST™ Binding/Wash Buffer

4.2mM	Na_2HPO_4
2mM	KH_2PO_4
140mM	NaCl
10mM	KCl

Elution Buffer

50mM	Glutathione (pH 7.0–8.0)
50mM	Tris-HCl (pH 8.1)

The provided Glutathione, 0.5M Solution, has a pH value between 7.0 and 8.0. If a different source of glutathione is being used, adjust the pH to 7.0–8.0 before adding the Tris-HCl (pH 8.1). The Glutathione, 0.5M Solution, has little buffering capacity at pH 7.0–8.0, so take care when adjusting the pH. Failure to adjust the pH of glutathione will decrease the pH of the elution bufffer, especially when final glutathione concentrations are ≥50mM.

9.C. Related Products

Product	Size	Cat.#
Single Step (KRX) Competent Cells	20 × 50µl	L3002
JM109 Competent Cells, >10^8cfu/µg	1ml	L2001
BL21(DE3)pLysS Competent Cells >10^6cfu/µg	1ml	L1191
Factor Xa Protease	50µg	V5581
Broad Range Protein Molecular Weight Markers	100 lanes	V8491
Gel Drying Kit, 17.5 × 20cm capacity	1 kit	V7120

9.C. Related Products (continued)

Product	Size	Cat.#
MagneHis™ Protein Purification System	65 reactions	V8500
	325 reactions	V8550
MagneHis™ Ni-Particles	2ml	V8560
	10ml	V8565
Magne™ HaloTag® Beads, 20% Slurry	1ml	G7281
	(5 × 1ml)	G7282
HaloLink™ Resin	1.25ml	G1912
	2.5ml	G1913
	25ml	G1915
HisLink™ Protein Purification Resin	50ml	V8821
MagneSphere® Technology Magnetic Separation Stand (twelve-position; 1.5ml)	1 each	Z5342
MagneSphere® Technology Magnetic Separation Stand (twelve-position; 12 × 75mm)	1 each	Z5343
MagneSphere® Technology Magnetic Separation Stand (two-position; 1.5ml)	1 each	Z5332
MagneSphere® Technology Magnetic Separation Stand (two-position; 12 × 75mm)	1 each	Z5333

9.D. Summary of Changes

The following change was made to the 8/14 revision of this document:

The composition of the MagneGST™ Binding/Wash Buffer was corrected to 2mM KH_2PO_4.

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