GST•Bind™ Kits

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* patent pending

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Europe

France	Germany	Ireland	United Kingdom	All other European Countries
Freephone 0800 126 461	Freecall 0800 100 3496	Toll Free 1800 409 445	Freephone 0800 622 935	+44 115 943 0840

techservice@merckbio.eu

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

www.novagen.com

About the Kits

Product	Size	Cat. No.
GST•Bind™ Buffer Kit		70534-3
GST•Bind Resin	10 ml	70541-3
	50 ml	70541-4
	25 ml	70541-5
BugBuster® GST•Bind Purification Kit		70794-3
GST•Mag™ Agarose Beads	2 ml	71084-3
	10 ml	71084-4
PopCulture® GST•Mag Purification Kit		71113-3

Description

GST•Bind™ Resin

GST•Bind™ Resin is an affinity chromatography support for rapid, single-step purification of recombinant glutathione S-transferase (GST) fusion proteins or native glutathione S-transferase or glutathione-binding proteins. GST•Tag™ fusion proteins containing the 220 aa GST domain (encoded by pET-41, pET-42, or pET-49 series vectors) are quickly and easily purified to near homogeneity in a single chromatographic step. Gentle elution with 10 mM reduced glutathione often preserves target protein activity.

GST•Bind Resin utilizes an 11-atom spacer arm (estimated 16 Å) for covalent attachment of reduced glutathione via a sulfide linkage. The high degree of substitution ensures excellent binding capacity, allowing yields of 5–8 mg GST•Tag fusion protein per 1 ml settled resin.

GST•Bind™ Buffer Kit

The GST•Bind Buffer Kit contains a set of pre-tested buffers for optimized binding, washing and elution of target proteins from GST•Bind Resin and GST•Mag™ Agarose Beads.

BugBuster® GST•Bind™ Purification Kit

The BugBuster® GST•Bind Purification Kit combines the GST•Bind Resin, GST•Bind Buffer Kit reagents, Benzonase® Nuclease and BugBuster Protein Extraction Reagent for convenient preparation of soluble cell extracts and affinity purification of GST•Tag fusion proteins. BugBuster reagent gently disrupts the cell wall of *E. coli* to release soluble protein. It provides a simple, rapid, low-cost alternative to mechanical cell lysis methods such as French press or sonication. The proprietary formulation includes a mixture of non-ionic detergents capable of perforating cell walls without denaturing soluble protein. Features of Benzonase Nuclease are described on p 4.

Note: *BugBuster is supplied in a Tris-HCl-based buffer. For applications requiring other buffers, Novagen® offers BugBuster Reagent (primary amine free), which contains a PIPPS buffer, and 10X BugBuster Reagent, which does not contain added buffer.*

GST•Mag™ Agarose Beads

GST•Mag Agarose Beads consist of reduced glutathione attached to 1–5 micron diameter magnetic agarose beads. High binding capacity allows purification of up to 2 mg GST•Tag fusion proteins per 1 ml settled resin. These magnetizable beads are ideal for rapid purification of multiple samples with miminal handling and are compatible with high-throughput applications. GST•Mag Agarose Beads are compatible with the GST•Bind Buffer Kit, BugBuster Protein Extraction Reagent, and PopCulture® Reagent.

PopCulture® GST•Mag Purification Kit

The PopCulture GST•Mag Purification Kit is ideally suited for high-throughput protein extraction and purification using GST•Mag Beads in the original culture tube or multiwell plate, without the need for centrifugation or columns. PopCulture Reagent is a Tris-buffered mixture of concentrated detergents that perforate the *E. coli* cell wall and extract proteins directly in the culture medium, without denaturating soluble protein (1). To further enhance purification, rLysozyme™ Solution is included in this kit; see p 5 for more information about use of this reagent.

Components

GST•Bind™ Buffer Kit

The GST•Bind™ Buffer Kit contains the following components sufficient to run a minimum of ten 2.5 ml columns:

- 2 × 100 ml 10X GST Bind/Wash Buffer (43 mM Na_2HPO_4, 14.7 mM KH_2PO_4, 1.37 M NaCl, 27 mM KCl, pH 7.3)
- 40 ml 10X Glutathione Reconstitution Buffer (500 mM Tris-HCl, pH 8.0)
- 1 g Glutathione, Reduced, Free Acid

BugBuster® GST•Bind Purification Kit

- 2 × 100 ml BugBuster® Protein Extraction Reagent
- 10,000 U Benzonase® Nuclease, Purity >90%
- 10 ml GST•Bind Resin, 10 ml settled volume (20 ml of a 50% v/v suspension)
- 4 Chromatography columns, with closures
- 100 ml 10X GST Bind/Wash Buffer
- 40 ml 10X Glutathione Reconstitution Buffer
- 1 g Glutathione, Reduced, Free Acid

GST•Mag™ Agarose Beads

- 2 × 1 ml or 10 × 1 ml GST•Mag™ Agarose Beads, 1 ml settled volume (2 ml of a 50% v/v suspension in 50 mM Phosphate Buffer, pH 7.5, containing 0.15 M NaCl and 0.1% NaN_3)

PopCulture® GST•Mag Purification Kit

- 15 ml PopCulture® Reagent
- 300 KU rLysozyme™ Solution
- 1 ml rLysozyme Dilution Buffer (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton® X-100)
- 3 × 1 ml GST•Mag Agarose Beads (2 ml of a 50% v/v suspension in 50 mM Phosphate Buffer, pH 7.5, containing 0.15 M NaCl and 0.1% NaN_3)
- 2 × 100 ml 10X GST Bind/Wash Buffer
- 40 ml 10X Glutathione Reconstitution Buffer
- 1 g Glutathione, Reduced, Free Acid

Storage

Store 10X GST Bind/Wash Buffer; 10X Glutathione Reconstitution Buffer; Glutathione, Reduced, Free Acid; GST•Bind Resin; and GST•Mag Agarose Beads at 4°C. Store rLysozyme Solution; rLysozyme Dilution Buffer; and Benzonase Nuclease at –20°C. Store BugBuster Reagent, PopCulture Reagent, and chromatography columns at room temperature. **DO NOT freeze GST•Bind Resin or GST•Mag Agarose Beads.**

Note: Storage of BugBuster Reagent and PopCulture Reagent at temperatures below 4°C may cause precipitation of the detergents. Incubate BugBuster Reagent or PopCulture Reagent in a room temperature water bath with gentle swirling or inversion to redissolve. Product performance is unaffected.

Note: Storage of rLysozyme Solution at –70°C may result in precipitation and loss of activity. Dilutions of rLysozyme Solution of 1:100 or less in the supplied Dilution Buffer are stable at 4°C for 1 week. Do not store diluted rLysozyme Solution at –20°C as this will cause activity loss and/or precipitation.

Overview

This User Protocol describes methods for *E. coli* cell extract preparation (mechanical disruption, BugBuster® Protein Extraction Reagent, and PopCulture® Reagent) and procedures for purification of GST•Tag™ target proteins using GST•Bind™ Resin or GST•Mag™ Agarose Beads. These procedures start with a cell culture that has been induced for target protein production. Target protein should bear the 220 amino acid schistosomal GST domain (GST•Tag sequence), which must be properly folded to allow interaction with immobilized reduced glutathione (see p 5). A detailed discussion of the induction of target proteins using Novagen's pET System can be found in the pET System Manual (User Protocol TB055).

Cell Extract Preparation

Growth and induction

Bacterial strain: Protein extraction is efficient for derivatives of both B (e.g., BL21) and K-12 (e.g., NovaBlue) strains. Strain-dependent differences in extraction efficiency are possible. Growth rate of the bacteria should be considered if performing the entire experiment in one day. In general, BL21 and its derivatives have a vigorous growth rate and are ideal for this extraction method. Lysozyme-expressing hosts bearing pLysS or pLysE plasmids increase protein extraction efficiency without addition of exogenous lysozyme (see p 5).

Cell Culture: The entire procedure from culturing through purification can be done in one day. It may be convenient to use small culture volumes ($\leq$50 ml) or multiwell plate cultures. For example, take a single colony or several colonies from a freshly streaked plate (with appropriate antibiotics) and inoculate 3 ml pre-warmed medium. After the OD_{600} reaches 0.8–1.0, inoculate 50 ml antibiotic-containing medium with the entire 3 ml culture. Let the 50 ml culture grow until OD_{600} reaches 0.8–1.0 and then induce. Use pre-warmed medium to avoid a lag in growth. Note that strains with a slower growth rate may be difficult to use in a single-day procedure. Alternatively, grow a culture overnight at reduced temperature (24–26°C) and induce the following day after raising temperature to 37°C and growing to an appropriate OD_{600} prior to induction.

Considerations before you begin

- BugBuster Reagent or PopCulture Reagent with Benzonase® Nuclease and rLysozyme™ Solution can greatly simplify extraction of soluble proteins and/or preparation of inclusion bodies. These reagents render mechanical disruption unnecessary; see below for more information.
- The GST•Bind and GST•Mag purification systems are compatible with additives such as 2-mercaptoethanol, dithiothreitol, and EDTA.
- BugBuster Reagent, PopCulture Reagent, Benzonase Nuclease, rLysozyme Solution, and both GST•Bind and GST•Mag systems are compatible with protease inhibitors. See p 5 for more information.
- The protocol can be scaled according to the expression level and amount of protein to be purified. See *GST•Bind Resin Chromatography* on p 8 for resin capacity.

Benzonase® Nuclease

Benzonase Nuclease (see User Protocol TB261) is a genetically engineered endonuclease from *Serratia marcescens* (2, 3) compatible with mechanical disruption, BugBuster Reagent, or PopCulture Reagent cell extract preparation. It degrades all forms of DNA and RNA. Nucleic acid polymers are degraded to 5'-monophosphate-terminated oligonucleotides 2–5 bases in length (4). This reduces lysate viscosity, permitting increased flow rates during chromatography. Use of Benzonase Nuclease is not recommended for preparing nuclease-free samples (5), as glutathione affinity purification may not remove all nuclease (however, subsequent anion exchange chromatography may remove nuclease). Benzonase Nuclease does not interfere with FRETWorks™ S•Tag™ Assay, the S•Tag Rapid Assay, or the GST•Tag Assay.

Benzonase Nuclease is inhibited (approximately 50% reduction in relative activity) by monovalent cations at >50 mm, phosphate at >20 mM, and by ammonium sulfate at >25 mM. Although Benzonase Nuclease requires Mg^{2+} for activation, no Mg^{2+} supplementation is required under the conditions described here. When handling small volumes, Benzonase Nuclease can be diluted with

50 mM Tris-HCl pH 8, 20 mM NaCl and 2 mM MgCl2. Diluted Benzonase® Nuclease can be stored at 4°C for several days without loss of activity.

rLysozyme™ Solution

Lysozyme cleaves a peptidoglycan bond in the *E. coli* cell wall, facilitating cell lysis and increasing protein yield (1, 6). rLysozyme™ Solution (see User Protocol TB334) is compatible with PopCulture® Reagent, BugBuster® Protein Extraction Reagent, and mechanical methods of cell extract preparation. Use of rLysozyme Solution is highly recommended to achieve complete cell lysis and release of large proteins. However, it is unnecessary when using hosts bearing pLysS or pLysE plasmids.

rLysozyme Solution may be diluted in rLysozyme Dilution Buffer, but dilutions should be used as soon as possible. Diluted rLysozyme Solution should not be stored at –20°C, as this will result in activity loss.

Protease Inhibitors

Protease inhibitors may be added to the buffers to protect against degradative enzymes. Serine protease inhibitors should be used with caution if the target protein is to be treated with thrombin, Factor Xa, or enterokinase, because any active inhibitor carried through the purification may affect cleavage reactions. If proteolytic degradation of the target protein is a problem, we suggest addition of: *p*-APMSF (10–100 µM; Calbiochem 178281); Pepstatin A (1 µM; Calbiochem 516481); Leupeptin (10–100 µM; Calbiochem 108975); Aprotinin (2 µg/ml; Calbiochem 616370); or Protease Inhibitor Cocktail Set II (with EDTA, Calbiochem 539132) or III (without EDTA, Calbiochem 539134). We recommend including a dialysis or gel filtration step prior to fusion tag cleavage with rEK, Factor Xa or thrombin. D-Tube™ Dialyzers are convenient dialysis devices; see User Protocols TB422 and TB495 for more information.

Inactive GST

Inactive GST will not bind GST•Bind™ Resin or GST•Mag™ Agarose Beads; only properly folded, functional GST is capable of binding. Target proteins with inactive GST cannot be used for glutathione affinity purification. GST•Tag™ fusion proteins present in inclusion bodies must be refolded to reconstitute active GST before using GST•Bind Resin or GST•Mag Agarose Beads. Reagents and a protocol for a simple, effective method of refolding are available in the Protein Refolding Kit (Cat. No. 70123-3). Note that active GST may not be obtained from all proteins refolded with the Protein Refolding Kit. The GST•Tag Assay (Cat. No. 70532-3) can be used to measure the amount of functional GST in any sample, enabling accurate calculation of theoretical maximum yield of purified target protein. (Alternatively, if the fusion protein also contains a His•Tag® sequence, it may be possible to solubilize inclusion bodies in 1X His•Bind® Binding Buffer containing 6 M urea or 6 M guanidine-HCl and purify using His•Bind resins under denaturing conditions; see User Protocols TB054 and TB273 for details.)

Mechanical disruption method

Soluble fraction - mechanical

This procedure isolates protein from the soluble fraction of *E. coli*. Prepare 1X GST Bind/Wash Buffer for the procedures below by diluting the 10X supplied stock with deionized water, or prepare according to buffer compositions given on page 3.

1. Harvest cells by centrifugation at 10,000 × *g* for 5 min. Decant supernatant and allow cell pellet to drain completely. Resuspend cells in 4 ml ice-cold 1X GST Bind/Wash Buffer per 100 ml culture. If desired, NP-40 or another non-ionic detergent can be added to a final concentration of 0.1% to reduce non-specific binding. A Dounce homogenizer, a blender or sonicator can be used to break up the cell pellet, if necessary.
2. Sonicate the sample in a tube on ice or in a salt-ice bath. Exact conditions are not specified here because results depend on type of sonicator probe used, power setting, and shape and size of the sample container. Avoid long sonication times; instead, use intermittant sonication bursts and cool sample on ice in between. Sonicate until the sample is no longer viscous. If DNA is not sheared, high extract viscosity will cause the column to clog. Large cell masses may be broken in 1X GST Bind/Wash buffer by alternative methods such as French press.

rLysozyme™ Solution (Cat. No. 71110-3) in combination with sonication facilitates efficient bacterial cell lysis.

Optional: Add rLysozyme Solution to a final concentration of 45–60 KU/gram of cell paste. Mix by pipeting. Incubate at 30°C for 15 min prior to sonication. See p 5 for more information on rLysozyme Solution.

3. Centrifuge lysate at 10,000 × *g* for 20 min. Filter the post-centrifugation supernatant though a 0.45 micron membrane. (Syringe-end filters are convenient). If samples are to be used with GST•Mag™ Agarose Beads, add Triton® X-100 to a final concentration of 0.5%, then clarify using a 0.45 micron membrane.

Inclusion body purification - mechanical

While some target proteins remain soluble in the cytoplasm, others form insoluble aggregates, or inclusion bodies, in *E. coli*. In addition to the target protein, inclusion bodies also contain contaminating bacterial proteins and nucleic acids. It may be possible to solubilize target protein from purified inclusion bodies and refold the protein prior to GST•Bind™ purification (see p 5).

1. Harvest the cells by centrifugation at 10,000 × *g* for 5 min. Decant supernatant and allow cell pellet to drain completely. Resuspend cells in 40 ml 1X GST Bind/Wash Buffer.
2. Sonicate briefly as described in Step 2 of *Soluble Fraction* section on p 5. This should resuspend the pellet thoroughly and shear DNA.
3. Centrifuge at 5,000 × *g* for 15 min to collect inclusion bodies and cellular debris.
4. Remove supernatant and suspend pellet in 20 ml 1X GST Bind/Wash Buffer per 100 ml culture volume. Repeat Step 3. Sonication may be necessary for resuspension. Note, however, that repeating this step several times can release more trapped contaminating proteins.

Note: To improve purity, rLysozyme™ Solution may be added at Step 4 (although it is not required). Add 1 KU/ml (final concentration) to the resuspended material in 1X GST Bind/Wash Buffer. Vortex gently, incubate for 5–10 min, and proceed with centrifugation.

5. Solubilize the washed inclusion bodies. Refold prior to glutathione affinity purification.

Cell extract preparation using BugBuster® Reagent

BugBuster® Protein Extraction Reagent allows a simple, rapid, non-mechanical method of lysing *E. coli* cells. Induced cells are harvested by centrifugation, resuspended in BugBuster Reagent, and incubated briefly at room temperature. Following centrifugation, the supernatant containing soluble protein is ready for GST•Bind purification. BugBuster Reagent can also be used to prepare highly purified IB prior to working with insoluble target proteins. Many different formulations of BugBuster Reagent are available; see User Protocol TB245 for more information.

General Considerations for BugBuster Protein Extraction Reagent

- BugBuster Reagent and Benzonase® Nuclease work most efficiently at room temperature.
- BugBuster Reagent can be used on fresh or frozen cell pellets. For comparisons of multiple samples (e.g., extended time course analysis), all cell pellets should be processed identically (either all fresh or all frozen). Superior extraction efficiencies may be obtained by freezing the cell pellet prior to resuspension in BugBuster Reagent. Multiple freeze-thaw cycles are not recommended because some target proteins may be inactivated.

Note: For optimal extraction of high molecular weight proteins (>70 kDa), we highly recommend freezing bacterial cell pellets prior to extraction with BugBuster Reagent and/or addition of rLysozyme Solution (see p 5).

- Extraction efficiency is somewhat strain-dependant and appears to be especially effective with BL21 and derivatives (BLR, Tuner™, Rosetta™, and Origami™ B strains).
- BugBuster Protein Extraction Reagent may be used in combination with Benzonase Nuclease and rLysozyme Solution.

Soluble fraction – BugBuster® Reagent

This fraction will consist of soluble protein present in both the periplasm and cytoplasm. If a separate periplasmic fraction is desired, first follow the osmotic shock procedure in the pET System Manual (see User Protocol TB055). The pellet from the osmotic shock procedure can be used in the protocol below.

1. Harvest cells from liquid culture by centrifugation at 10,000 × *g* for 10 min using a centrifuge tube of known weight. For small-scale extractions (1.5 ml or less), centrifugation can be performed in a 1.5 ml tube at 14,000–16,000 × *g*. Decant and allow pellet to drain, removing as much liquid as possible. Determine wet weight of the pellet.
2. Resuspend cell pellet in room-temperature BugBuster Reagent by pipeting or gentle vortexing. Use 5 ml reagent per g wet cell paste. For small cultures use up to 1/5 culture volume for

resuspension (e.g., use 300 µl BugBuster® for 1.5 ml cultures). There are no adverse effects caused by using larger volumes of BugBuster Reagent.

3. Add 1 KU rLysozyme™ Solution per 1 ml BugBuster Reagent (5 KU/g cell paste).
   Optional:
   a) Add 1 µl (25 units) Benzonase® Nuclease per 1 ml of BugBuster Reagent.
   b) Add protease inhibitors. See p 5 for more information.
4. Incubate on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature. If using Benzonase Nuclease, extract should not be viscous at the end of the incubation.
5. Remove insoluble cell debris by centrifugation at 16,000 × *g* for 20 min at 4°C. If desired, save pellet for inclusion body purification as described below.
6. Transfer supernatant to a fresh tube. Soluble extract can be applied directly to GST•Bind™ Resin or GST•Mag™ Agarose Beads. Maintain clarified extracts on ice for short-term storage (2–3 h) or store at 4°C. If detergents in the extract precipitate upon storage, gently swirl and/or invert at room temperature to redissolve before applying to purification resin. Freezing the extract is not recommended.

Inclusion body purification – BugBuster Reagent

4. Process induced culture according to Steps 1–4 above for the soluble protein fraction.
5. Resuspend pellet from step 4 above in the same volume of BugBuster Reagent used to resuspend the original cell pellet. Pipet sample and vortex to mix. Thorough resuspension of the pellet is critical for optimal purity.
6. Add rLysozyme Solution to a final concentration of 1 KU/ml. Vortex gently to mix. Incubate at room temperature for 5–10 min.
7. Add 6 volumes diluted BugBuster reagent (diluted 1:10 in deionized water) to the suspension. Vortex for 1 min.
8. Centrifuge suspension at 5,000 × *g* for 15 min at 4°C to collect inclusion bodies. Remove supernatant using a pipet.
9. Resuspend the inclusion bodies in half the original culture volume of 1:10 diluted BugBuster Reagent, mix by vortexing, and centrifuge as in Step 5. Repeat this wash step twice more, for a total of 3 washes. After the final resuspension, centrifuge at 16,000 × *g* for 15 min at 4°C. Remove the supernatant.
10. Resuspend the washed inclusion body pellet in buffer of your choice. GST•Tag™ fusion proteins must be refolded to reconstitute active GST before affinity purification (see p 5).

Cell extract preparation using PopCulture® Reagent

PopCulture® Reagent efficiently extracts protein from *E. coli* directly in culture medium without harvesting cells. An induced culture of *E. coli* may be treated with PopCulture Reagent for 10 min at room temperature. Proteins in this extract can be assayed directly. GST•Tag proteins can be purified by adding equilibrated GST•Bind Resin or GST•Mag Agarose. For optimal cell lysis and protein extraction, rLysozyme Solution and/or Benzonase Nuclease may be used. For more information on these additives, see p 4–5.

1. Culture E. coli bearing an expression plasmid, using optimal conditions for target protein expression. See section entitled Growth and Induction on p 4.
2. Add 0.1 culture volume of PopCulture Reagent.
3. Add 40 U (1 µl of a 1:750 dilution) rLysozyme Solution per 1 ml of original culture volume.
   Optional:
   a) Add 1 µl (25 U) Benzonase Nuclease per 1 ml of original culture volume.
   b) rLysozyme Solution and Benzonase Nuclease can be pre-mixed with PopCulture Reagent to facilitate rapid sample processing. Pre-mixed PopCulture Reagent, rLysozyme Solution and Benzonase Nuclease should be used the same day, and can be stored at 4°C no longer than 1 day.
4. Pipet sample to mix thoroughly. Incubate for 10–15 min at room temperature.
5. After PopCulture Reagent incubation is complete, extract can be assayed directly and/or combined with equilibrated GST•Bind Resin or GST•Mag Agarose Beads. Follow standard resin-specific protein purification procedures, as described below. Highly-expressed target

protein may be visualized by mixing with 4X SDS Sample Buffer (Cat. No. 70607-3) and loading the maximum possible volume on a lane of an SDS-PAGE gel.

Additional guidelines for PopCulture® Reagent optimization and compatibility

Benzonase® Nuclease and rLysozyme™ Solution: PopCulture® Reagent is compatible with rLysozyme Solution and Benzonase Nuclease.

Volume of PopCulture® Reagent: There are no adverse effects caused by using higher ratios of PopCulture Reagent. If the cells need to be concentrated prior to extraction, the volume of PopCulture Reagent can be adjusted accordingly.

Temperature of extraction: Extraction using PopCulture Reagent along with rLysozyme Solution and Benzonase Nuclease can be performed at room temperature or at 4°C. However, incubation times may need to be increased, because rLysozyme Solution and Benzonase Nuclease activities decrease at lower temperatures.

pH of extraction: Acidic pH (<5.0) can degrade components of PopCulture Reagent.

Protein assays: Protein-specific activity assays and immunoassays are likely to be compatible with extraction using PopCulture Reagent. PopCulture Reagent is compatible with the GST•Tag™ Assay, S•Tag™ Rapid Assay and FRETWorks™ S•Tag Assay Kits, as well as protein quantification assays including BCA (Cat. No. 71285) and Coomassie-based assays.

Protease inhibitors: Protease inhibitors may be added to the culture medium along with PopCulture Reagent. See p 5 for more information.

GST•Bind™ Resin Chromatography

Optimal results with GST•Bind™ Resin are usually obtained by using a volume of resin with binding capacity 10–20% in excess over the amount of target protein in the extract sample. The capacity of GST•Bind Resin is approximately 5–8 mg/ml settled resin. A yield of 20 mg of target protein per 100 ml culture is not unusual with the pET system. However, target protein expression levels vary considerably depending on the gene sequence, vector construct, host strain, and growth and induction conditions. SDS-PAGE or Western blotting allow rough quantification of target protein in crude extracts. More accurate quantification can be obtained using the S•Tag Rapid Assay (Cat. No. 69212-3) or FRETWorks S•Tag Assay (Cat. No. 70724-3) if the target protein also bears an S•Tag peptide. Other protein-specific assays can likewise be used. For proteins with a GST•Tag, the most reliable data are obtained using the GST•Tag Assay (Cat. No. 70532-3), which measures the amount of functional GST in the extract.

Buffer preparation

1. Dilute an appropriate volume of the supplied stock of 10X GST Bind/Wash Buffer to a 1X final concentration with sterile deionized water. Approximately 15 volumes of GST Bind/Wash Buffer will be needed for column chromatography, 26 volumes for batch purification from extracts without medium (mechanical or BugBuster® Reagent method), or 51 volumes for batch purification from extracts with medium (PopCulture Reagent method). One volume is equivalent to the settled bed volume (e.g., 100 µl of slurry yields 50 µl of resin for a settled bed volume of 50 µl).
2. Prepare 10X GST Elution Buffer containing 100 mM reduced glutathione by dissolving 1 g reduced glutathione in 32.5 ml 10X Glutathione Reconstitution Buffer. After 10X GST Elution Buffer is prepared, it must be divided into working volumes and stored at –20°C to prevent oxidation of the glutathione. 10X GST Elution Buffer is stable at –20°C for 6 months with no more than 5 freeze/thaw cycles. Immediately before use, dilute 10X GST Elution Buffer to 1X with sterile deionized water. Approximately 3 volumes of 1X GST Elution Buffer will be needed for column chromatography or batch purification from extracts without medium (mechanical or BugBuster Reagent method) or 2–6 volumes for batch purification from extracts containing medium (PopCulture Reagent method).

Note: 1X GST Elution Buffer must be prepared fresh immediately before use to prevent oxidation of the glutathione.

Column chromatography

Perform purification at room temperature. Allow resin and buffer components to equilibrate to room temperature before use. GST•Bind™ Resin can be used in either column format (below) or batch format (see p 9).

1. Gently mix the bottle of GST•Bind Resin by inversion until completely suspended. Using a wide-mouth pipet, transfer desired amount of slurry to a column (remember that resin is supplied as a 50% slurry). To prevent introduction of air bubbles, keep pipet tip in contact with interior surface of column while transferring resin. Allow resin to settle. Small polypropylene columns (Cat. No. 69673-3) hold 2.5 ml of settled resin and allow convenient purification of up to 12–20 mg target protein.
2. When level of storage buffer (20% ethanol) drops to top of the column bed, wash resin with 5 volumes 1X GST Bind/Wash Buffer.

Note: *Immediately before loading the column, bring extract to room temperature rapidly using a room-temperature water bath.*

3. Allow GST Bind/Wash Buffer to drain to the top of the column bed and load column with prepared extract. A flow rate of about 10 column volumes per hour is optimal. If flow rate is too fast, more impurities will contaminate the eluted fraction. Collect flowthrough fraction and store on ice.
4. Wash column with 10 volumes 1X GST Bind/Wash Buffer. Collect flowthrough fraction and store on ice.
5. Elute bound protein with 3 volumes 1X GST Elution Buffer. Collect elution fractions for subsequent analysis and store on ice.
6. Analyze flowthrough and elution fractions for presence of target protein. Target protein that does not have functional GST will not bind resin.

Small scale purification - batch method

Extract without Medium – Prepared by Mechanical Lysis or BugBuster® Reagent

Settled bed volumes of 50 µl to 200 µl can be handled in 1.5 ml microcentrifuge tubes. For larger volumes, use 15 ml or 50 ml sterile disposable centrifuge tubes. For each wash, add buffer, invert tube several times to mix, and centrifuge for 1–5 min at 400–1000 × *g*.

1. Gently mix the bottle of GST•Bind Resin by inversion until completely suspended. Using a wide-mouth pipet, transfer desired amount of slurry to a centrifuge tube (remember that resin is supplied as a 50% slurry).
2. Pellet resin by centrifugation as described above. Carefully remove and discard supernatant. Add 5 volumes 1X GST Bind/Wash Buffer, invert to mix, and centrifuge as before.
3. Discard supernatant and add 1 volume 1X GST Bind/Wash Buffer. Resuspend resin (now a 50% slurry) by inversion.
4. Add protein sample and incubate mixture at room temperature for 30 min with gentle agitation.
5. Centrifuge for 1–5 min at 400–1,000 × *g* and transfer supernatant containing unbound proteins to a fresh tube. Store this supernatant on ice. Resuspend resin in 10 volumes 1X GST Bind/Wash Buffer, centrifuge, and remove supernatant. Store wash supernatant on ice and repeat, for a total of 2 washes.
6. Elute bound protein from pellet resin by adding 1 volume 1X GST Elution Buffer. Incubate at room temperature with gentle agitation for 10 min. Centrifuge and transfer supernatant containing the purified fusion protein to a fresh tube. Repeat the elution twice more, each time with 1 volume 1X GST Elution Buffer. Supernatants may be pooled if desired.
7. Analyze eluted fraction and supernatant from Step 5 for the presence of target protein. Target protein that does not have functional GST will not bind resin.

Extracts Containing Medium – Prepared with PopCulture® Reagent

A settled bed volume of 50 µl is recommended per 1 ml of original culture volume. For each wash step, add appropriate buffer and remove supernatant by centrifuging for 1–5 min at 400–1000 × *g*. In the following protocol, one volume is equivalent to the settled bed volume (e.g., 100 µl slurry yields settled bed volume of 50 µl resin).

1. Gently mix the bottle of GST•Bind™ Resin by inversion until completely suspended. Using a wide-mouth pipet, transfer desired amount of slurry to a centrifuge tube (remember that resin is supplied as a 50% slurry).
2. Pellet resin by centrifugation, as described above. Carefully remove the supernatant and discard. Add 5 volumes 1X GST Bind/Wash Buffer. Invert to mix and centrifuge as before.
3. Discard the final supernatant and add 1 volume 1X GST Bind/Wash Buffer. Resuspend resin (now a 50% slurry) by inversion.
4. Add equilibrated resin to the prepared extract. Mix gently by pipetting. Incubate for 5 min with mixing.

Note: As an alternative to batch method, the sample can be processed using a vacuum filter plate system or transferred to a 0.5 cm diameter column.

5. Centrifuge and transfer supernatant containing unbound proteins to a fresh tube and store on ice. Resuspend resin in 40 volumes 1X GST Bind/Wash buffer, centrifuge, and remove supernatant. Store supernatant on ice and repeat the wash steps, for a total of 2 washes.
6. Elute bound protein by adding 1–3 volumes 1X GST Elution Buffer. Incubate at room temperature with gentle agitation for 5 min. Transfer the supernatant, which contains purified fusion protein, to a fresh tube.
   Optional: Repeat the elution again with 1–3 volumes 1X GST Elution Buffer and pool the supernatants. Larger elution volumes may recover more target protein.
7. Analyze eluted fraction from Step 5 for presence of target protein. Target protein that does not have functional GST will not bind GST•Bind Resin.

Regeneration and storage

GST•Bind Resin can be reused several times without regeneration. However, accumulation of non-specifically bound proteins or protein aggregates may cause reduced flow rates or binding capacity. To remove bound proteins, resin should be washed with 10 volumes 50 mM Tris-HCl, 0.5 M NaCl, pH 8.0; followed by 10 volumes of 100 mM sodium acetate pH 4.5, 0.5 M NaCl. Other buffers that may remove column contaminants include: 6 M urea, 6 M guanidine-HCl, or low polarity solvents such as 50–70% ethanol or 50% ethylene glycol. Any of these treatments should be followed immediately by full equilibration with 10 column volumes of 1X GST Bind/Wash Buffer. For long-term storage, resin should be equilibrated in 20% ethanol/80% water and stored at 4°C.

GST•Mag™ Agarose Beads

Estimate the quantity of target protein in the extract, and then prepare a corresponding amount of GST•Mag™ Agarose Beads. The capacity of the magnetic beads is approximately 2 mg/ml settled beads. SDS-PAGE or Western blotting allow rough quantification of target protein in crude extracts. More accurate quantification can be obtained using the S•Tag™ Rapid Assay (Cat. No. 69212-3) or FRETWorks™ S•Tag Assay (Cat. No. 70724-3) if the target protein also bears an S•Tag peptide. Other protein-specific assays can likewise be used. For proteins with a GST•Tag™, the most reliable data are obtained using the GST•Tag Assay (Cat. No. 70532-3), which measures the amount of functional GST in the extract.

Buffer preparation

Note: When using GST•Mag Agarose Beads, add Triton® X-100 to a final concentration of 0.5% in 1X GST Bind/Wash Buffer and 1X GST Elution Buffer. If mechanical lysis methods (French press or sonication) are used to prepare lysate, add Triton X-100 to a final concentration of 0.5% to lysate before adding to beads. Use of Triton X-100 (0.5%) during purification reduces non-specific binding to the GST•Mag agarose beads and facilitates magnetic particle handling.

1. Dilute an appropriate amount of 10X GST Bind/Wash Buffer to 1X with sterile deionized water. Add Triton X-100 to a final concentration of 0.5%. Approximately 42 volumes GST Bind/Wash Buffer are needed for batch purification from extracts without medium (mechanical lysis or BugBuster® reagent method) or 73–93 volumes for batch purification from extracts with medium (PopCulture® Reagent method). One volume is equivalent to the settled bed volume (e.g., 100 µl of slurry yields 50 µl of beads for a settled bed volume of 50 µl).
2. Prepare 10X GST Elution Buffer containing 750 mM reduced glutathione by dissolving 1 g reduced glutathione in 4.33 ml 10X Glutathione Reconstitution Buffer. Once 10X GST Elution

Buffer is prepared, it must be divided into working volumes and stored at –20°C to prevent oxidation of the glutathione. 10X GST Elution Buffer is stable at –20°C for 6 months with no more than 5 freeze/thaw cycles. Immediately before use, dilute 10X GST Elution Buffer to 1X with sterile deionized water. Add Triton® X-100 to a final concentration of 0.5%. Between 4–8 volumes of 1X GST Elution Buffer for purification from extracts without medium (mechanical lysis or BugBuster® Reagent) or from extracts containing medium (PopCulture® Reagent) will be needed.

Note: 1X GST Elution Buffer must be prepared fresh immediately before use to prevent oxidation of the glutathione.

GST•Mag Agarose Beads purification

Extracts without Medium – Prepared by Mechanical Lysis or BugBuster® Reagent

Perform purification at room temperature. Allow magnetic beads and buffer components to equilibrate to room temperature before use.

1. Gently mix the bottle of GST•Mag™ Agarose Beads by inversion until completely suspended. Transfer desired amount of slurry to a 1.5 ml, 15 ml or 50 ml tube. Place tube in a Magnetight™ rack or stand (Cat. No. 69964-3 or 70747-3) to collect beads.
2. Remove supernatant. Wash beads 3 times with 4 volumes of 1X GST Bind/Wash Buffer. For each wash, remove tube from the magnetic rack to resuspend beads with buffer, and then replace in magnetic rack to remove the buffer.

Note: Immediately before adding extract to the beads, bring the extract to room temperature rapidly using a room-temperature water bath. If mechanical lysis methods (French press or sonication) were used to prepare cell extract, add Triton X-100 to a final concentration of 0.5%. Clarifying the sample using a 0.45 micron filter before applying to beads can improve results.

3. After the final wash, resuspend beads in 1 volume 1X GST Bind/Wash Buffer to make a 50% slurry.
4. Combine equilibrated beads with prepared cell extract. Mix well by inverting several times. Incubate on a shaking platform for 10–20 min.
5. Place tube in a magnetic rack to collect beads and remove supernatant. Store supernatant on ice and retain for further analysis.
6. Wash beads 3 times with 8 volumes 1X GST Bind/Wash Buffer. For each wash, remove tube from the magnetic rack to resuspend beads with buffer, then replace in the magnetic rack to remove buffer. Store supernatant on ice and retain for further analysis.
7. Elute bound protein with 2–4 volumes 1X GST Elution Buffer. After removing tube from the magnetic rack, resuspend beads and incubate for 10 min with occasional mixing by tapping bottom of the tube. Do not invert tube.
8. Place tube in a magnetic rack to collect the beads. Remove eluted protein to a fresh tube.

   Optional: Repeat with an additional 2–4 volumes 1X GST Elution Buffer. Pool the supernatants.

Note: Typically, 3 volumes 1X Elution Buffer are sufficient for elution, but the volume may be adjusted as needed.

9. Analyze eluted fraction and supernatant from Step 5 for presence of target protein.

Extracts Containing Medium – Prepared with PopCulture® Reagent

For each 1 ml of original culture prepared with PopCulture Reagent, use 50 µl of a 50% slurry of GST•Mag Beads. Bead volume may be adjusted according to yield estimates. Perform purification at room temperature. Allow magnetic beads and buffer components to equilibrate to room temperature before use.

1. Gently mix the bottle of GST•Mag Agarose Beads by inversion until completely suspended. Transfer an appropriate amount of resuspended GST•Mag Agarose Beads to a tube or multiwell plate. Place tube in a Magnetight rack (Cat. No. 69964-3 or 70747-3) or other magnetic device to collect the beads.
2. Remove supernatant and wash beads 3 times with 4 volumes 1X GST Bind/Wash Buffer. For each wash, remove tube from the magnetic rack to resuspend beads with buffer and then replace in magnetic rack to remove buffer.

Note: Immediately before adding extract to the beads, bring extract to room temperature rapidly using a room-temperature water bath. After the final wash, resuspend beads in 1 volume 1X GST Bind/Wash Buffer.

3. Combine equilibrated beads with prepared cell extract. Incubate for 5 min with mixing.
4. Remove supernatant and store on ice.
5. Wash beads 3 times with 20–30 volumes 1X GST Bind/Wash Buffer. For each wash, remove the sample from the magnetic rack to resuspend the beads, then replace in magnetic rack to remove buffer.
6. Elute bound protein with 2–4 volumes 1X GST Elution Buffer. Incubate for 5 min and remove supernatant.
   Optional: Repeat with an additional 2–4 volumes 1X GST Elution Buffer. Pool supernatants.
7. Analyze eluted fraction and supernatant from Step 5 for presence of target protein.

Processing Sample after Elution

After eluting purified protein from GST•Bind™ Resin or GST•Mag™ Agarose Beads, the sample can be concentrated or the buffer exchanged as necessary. D-Tube™ Dialyzers are convenient for both dialysis and concentration in a single-tube format, and are available in a variety of volume capacities and molecular weight cutoffs (MWCO). See User Protocols TB422 and TB495 for more information. Appropriate buffer and storage conditions should be determined empirically, as many proteins are prone to degradation and/or aggregation upon long-term storage (7).

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Troubleshooting guide

Problem	Probable Cause	Solution
No binding or inefficient binding to GST•Bind™ Resin or GST•Mag™ Agarose Beads	GST•Tag™ sequence not present	Sequence ligation junctions to verify correct reading frame. Use protease-deficient *E. coli* strains. Use protease inhibitors. Use Rosetta™ strain if protein contains rare codons.
	GST fusion protein denatured by sonication	Extensive sonication can denature tagged protein and prevent it from binding to GST•Bind Resin or GST•Mag Agarose Beads. Use mild sonication conditions during cell lysis, or use BugBuster® Protein Extraction Reagent.
	Incorrect binding conditions	Check pH and composition of all buffers and solutions. Binding of GST-tagged proteins to GST•Bind Resin or GST•Mag Agarose Beads is not efficient at pH below 6.5 or above 8. Make sure resin has been equilibrated with a buffer in pH range 6.5–8.0 (e.g., 1X GST Bind/Wash buffer, PBS) before applying cell lysate. Adding DTT prior to cell lysis may significantly increase binding of some GST fusion proteins to GST•Bind Resin or GST•Mag Agarose Beads. Adding Lysonase™ or rLysozyme™ prior to sonication may improve results.
	GST•Bind Resin has been used numerous times	If the GST•Bind Resin has been re-used several times, regeneration or replacement may be necessary.
Protein does not elute, or elutes inefficiently	Low elution volume	Occasionally, a larger volume of buffer may be necessary to elute the target protein.
	Insufficient concentration of glutathione in the elution buffer	Reduced glutathione at a concentration of 10 mM should be sufficient with GST•Bind resin. However, higher concentrations (up to 75 mM reduced glutathione) can be used for elution.
	Incorrect pH of the elution buffer	Low pH may limit elution from column. Increasing the pH of elution buffer to pH 9 may improve elution without requiring an increase in the concentration of reduced glutathione.
	Low ionic strength of the elution buffer	Adding 0.1–0.2 M NaCl to the elution buffer may improve results.
	Elution buffer is old	10X GST Elution Buffer is stable at –20°C for 6 months with a maximum of 5 freeze/thaw cycles. Prepare fresh 1X elution buffer immediately before use.
	Degradation of GST•Tag fusion protein	Add protease inhibitor(s) to lysis solution. Multiple bands may be a result of partial degradation of tagged proteins by proteases. Use a protease-deficient host. Multiple bands may result from proteolysis in the host bacteria. A protease deficient (*lon* or *ompT*) host strain may be required.
Protein elutes with contaminants	Target protein is not full length	Use Rosetta strain if cDNA contains rare codons.
	Excessive sonication	Decrease sonication. Avoid frothing as this may denature fusion protein. Over-sonication can also lead to co-purification of host proteins with GST fusion protein.
	Co-purification of chaperonins	Additional bands may be caused by co-purification of chaperonins, which facilitate correct nacent protein folding. These include: DnaK (70 kDa), DnaJ (37 kDa), GrpE (40 kDa), GroEL (57 kDa), and GroES (10 kDa). An additional purification step can be included.
Slow flow rate	Cellular debris in sample	Filter sample through 0.45 micron low protein-binding filter to remove debris prior to purification. Refer to *Regeneration and Storage* (p 10).