GE Healthcare

GST Bulk Kit

Product Booklet

Code: 27-4570-01

Page finder

1. Legal

http://www.gelifesciences.com/sampleprep

GE Healthcare UK Limited.
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2. Handling

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at 4–8°C.
Do not freeze.

3. Components of the kit

GST Bulk kit contains

10 ml bulk pack of Glutathione Sepharose™ 4B.

5 disposible columns.

GST buffer kit.

500 mg Isopropyl β-D Thiogalactoside.

4. Introduction

GST Bulk Kit contains 10 ml bulk pack of Glutathione Sepharose 4B medium and five disposable columns. It is designed for small-scale manual purification of GST-tagged proteins in batch experiments or with packed columns using a gravity flow column format. GST Bulk Kit facilitate optimization studies of expression, solubility conditions and purification parameters of a GST-tagged protein. One kit is suited for purification of GST-tagged proteins from 2 ml to 20 l of culture in up to five columns in parallel (up to 100 mg purified GST-tagged proteins).

Glutathione Sepharose 4B medium is designed for purification of recombinant derivatives of glutathione S-transferase (GST), other glutathione S-transferases or glutathione dependent proteins. The capacity will vary with the nature of the tagged protein and the binding conditions used. For increased convenience GST Bulk Kit contains all buffers needed for purification of GST-tagged proteins. GST-tagged proteins are recovered from the matrix under mild elution conditions (10 mM glutathione) which preserve functionality of the proteins. The GST tag is originated from the different pGEX vectors provided by GE healthcare (see ordering information). The pGEX vectors are designed for inducible, high-level intracellular expression of genes or gene fragments as fusions with *Schistosoma japonicum* GST.

5. Principle and characteristics

Glutathione Sepharose 4B medium,has high binding capacity for GST-tagged proteins. Table 1 summarizes Glutathione Sepharose 4B medium and disposable column characteristics.

Table 1. Glutathione Sepharose 4B medium and disposable column characteristics.

Column material	Polypropylene barrel, polyethylene frits
Column volume	5 ml
Medium	Glutathione Sepharose 4B
Bead size	45–165 µm (average bead size 90 µm)
Ligand	glutathione and 10-carbon linker arm
Ligand concentration	7–15 µmol glutathione/ml medium
Protein binding capacity[1]	Approx. 10 mg recombinant glutathione S-transferase (M_r 26 000)/ml medium (protein dependent)
Recommended Bed volume	1 ml
Compatibility during use	All commonly used aqueous buffers
Chemical stability	No significant loss of the capacity is detected when Glutathione Sepharose 4B is exposed to 0.1 M citrate (pH 4.0), 0.1 M NaOH, 70% ethanol or 6 M guanidine hydrochloride [2] for 2 h at room temperature. No significant loss of binding capacity is observed after exposure to 1% SDS for 14 days.
Storage solution	20% Ethanol

pH stability	4 to 13
Storage temperature	4–30°C

Note: It is not recommended to autoclave the gel.

[1] Binding capacity is protein-dependent. The binding of GST-tagged proteins depends on size, conformation, and concentration of the protein in the sample loaded. Binding of GST to glutathione is also flow dependent, and lower flow rates often increase the binding capacity. This is important during sample loading. Protein characteristics, pH, and temperature may also affect the binding capacity.

[2] Exposing with 6 M guanidine hydrochloride will denaturate the GST-tag. It is therefore important to remove all guanidine hydrochloride before use.

6. GST Buffer Kit

Sufficient reagents are supplied to purify up to 20 mg of GST fusion protein, and it can be used with a variety of formats.

GST buffer kit content

- 50 ml 10 × PBS stock solution pH 7.4 (1.4 M NaCl, 27 mM KCl, 101 mM Na_2HPO_4, 18 mM KH_2PO_4, pH 7.4).
- 50 ml dilution buffer: 50 mM Tris-HCl (pH 8.0).
- Reduced Glutathione 0.154 g.

Buffer conditions for purification

Binding buffer: 10 mM PBS pH 7.4 (dilute stock solution 10 times which gives 140 M NaCl, 0.027 mM KCl, 10 mM Na_2HPO_4, 0.018 mM KH_2PO_4, pH 7.4)

Elution buffer: 50 mM Tris-HCl (pH 8.0), 10 mM glutathione (Addition of the 50 ml of dilution buffer to bottle containing glutathione)

7. Sample pretreatment

Cell lysis

For larger-scale cultures, mechanical lysis with either sonication or homogenizers is the most recommended methods to lyse cells. However, the process is only efficient for cell suspensions greater than 2 ml. While screening of multiple samples can be performed on aliquots of such larger lysis, the growth and processing of a large number of cultures of this size may be unwieldy. Chemical lysis with commercial lysis kits is a third alternative and simplify when lysis of multiple samples needs to be purified. There are several commercial lysis kits on the market with variable efficiency and lysis should be performed according to protocols for each method. GE Healthcare can provide lysis kits to two expression systems, Mammalian Protein Extraction Buffer for mammalian expression systems and Yeast Protein Extraction Buffer Kit for yeast expression systems. For bacteria there are several chemical lysis kits available on the market. For small-scale cultures, chemical lysis with commercial lysis kits and freeze/thaw are recommended to be used as lysis methods.

8. Purification protocol

The following protocol can be conveniently scaled to purify as little as 50 µg or as much as 50 mg of GST-tagged protein using the GST Bulk Kit. Yield of tagged protein is highly variable and is affected by the nature of the tagged protein, the host cell, and the culture conditions used. GST-tagged protein yields can range from 1–10 mg/l culture. Protocols are based on approximate culture volumes with an average yield of 2.5 mg/l.

Note: The yield of GST-tagged protein can be estimated by measuring the absorbance at 280 nm. The GST affinity tag can be approximated by 1 $A_{280} \cong 0.5$ mg/ml. This calculation is based on the extinction coefficient of the GST monomer using a Bradford protein assay. Other protein determination methods may produce different extinction coefficients.

The GST Bulk kit may be used for batch purification or to pack columns included with the kit with variable bed volumes of matrix.

Note! Bed volume is equal to 0.5 × the volume of the 50% Glutathione Sepharose slurry used or 0.75 × the volume of the original Glutathione Sepharose slurry.

8.1 Packing disposable gravity column with Glutathione Sepharose 4B

1. Remove the top cap from a disposable column included with the GSTBulk kit for each purification and place upright in an appropriate rack/clamp.
2. Determine the bed volume of Glutathione Sepharose 4B required for your application depending on how much protein that should be purified (~ 10 mg GST-tagged protein/ml Glutathione Sepharose 4B medium).

3. Gently shake the bottle of Glutathione Sepharose 4B to resuspend the matrix.
4. Use a pipette to remove sufficient slurry for use and transfer to the disposable column. (Glutathione Sepharose 4B as supplied is approximately a 75% slurry). Based on the bed volume requirements dispense 1.33 ml of the original Glutathione Sepharose 4B slurry per ml of bed volume required.)
5. Tap the column to dislodge any trapped air bubbles in the matrix bed. Allow to settle.
6. Remove the bottom cap and save for later use. Allow the column to drain.

Note: Gentle pressure with a gloved thumb over the top of the column may be required to start the flow of liquid.

7. Wash the Glutathione Sepharose 4B by adding 5 ml of cold (4–8°C) binding buffer (10 mM PBS pH 7.4) per 1.33 ml of the original slurry of Glutathione Sepharose 4B dispensed. Allow the column to drain.
8. If the column will be stored add the bottom tip and the top cap.

Note: Glutathione Sepharose 4B must be thoroughly washed with binding buffer to remove the 20% ethanol storage solution. Residual ethanol may interfere with subsequent procedures.

Note: Glutathione Sepharose 4B equilibrated with 10 mM PBS pH 7.4 may be stored at 4–8°C for up to 1 month.

8.2 purification with packed Gravity columns

1. Remove the bottom tip. Remove the top cap and pour of the excess liquid.
2. Wash the column with 5 bed volumes of binding buffer (10 mM PBS pH 7.4) to remove the preservative.

3. Apply the sample to the column. If needed, clarify the sample by centrifugation. The sample may be diluted in binding buffer if too concentrated.
4. Wash the column with 2 × 5 bed volumes of binding buffer.
5. Elute the bound material with 5 bed volumes of elution buffer (10 mM Glutathione in 50 mM Tris-HCl pH 8.0) and collect 1–2 ml fractions.

Note: Following the elution steps, a significant amount of GST-tagged protein may remain bound to the matrix. Volumes and times used for elution may vary among GST-tagged proteins. Additional elutions may be required. Eluates should be monitored for GST tagged protein by SDS-PAGE or by CDNB assay (GST Detection Module, 27-4590-01).

Regeneration and storage

The gel may be regenerated by washing the column with high salt buffer (PBS + 3 M NaCl. For longer storage, wash the column with 2 × 5 bed volumes of PBS and the 2 × 5 bed volumes of 20% ethanol. Store at 4–30°C.

8.3 Batch purification of GST-tagged proteins using bulk of Glutathione Sepharose 4B

1. Determine the bed volume of Glutathione Sepharose 4B required for your application (~ 10 mg GST-tagged protein/ml Glutathione Sepharose 4B medium).
2. Gently shake the bottle of Glutathione Sepharose 4B to resuspend the matrix.
3. Use a pipette to remove sufficient slurry for use and transfer to an appropriate container/tube. (Glutathione Sepharose 4B as supplied is approximately a 75% slurry. The following procedure results in 50% slurry. Based on the bed volume requirements, dispense 1.33 ml of the original Glutathione Sepharose 4B slurry per ml of bed volume required.)

4. Sediment the matrix by centrifugation at 500 × g for 5 mins. Carefully decant the supernatant.
5. Wash the Glutathione Sepharose 4B by adding 5 ml of binding buffer (10 mM PBS pH 7.4) per 1.33 ml of the original slurry of Glutathione Sepharose 4B dispensed. Invert to mix.

Note: Glutathione Sepharose 4B must be thoroughly washed with binding buffer to remove the 20% ethanol storage solution. Residual ethanol may interfere with subsequent procedures.

6. Sediment the matrix by centrifugation at 500 × g for 5 mins. Decant the supernatant.
7. For each 1.33 ml of the original slurry of Glutathione Sepharose 4B dispensed, add 1 ml of binding buffer. This produces a 50% slurry. Mix well prior to subsequent steps in the protocol.

Note: Glutathione Sepharose 4B equilibrated with 10 mM PBS pH 7.4 may be stored at 4°C for up to 1 month.

Batch binding/column wash

1. Add 2 ml of the 50% slurry of Glutathione Sepharose 4B equilibrated with binding buffer to each 100 ml of lysed sample.
2. Incubate with gentle agitation at room temperature for 30 mins.
3. Use a pipette to transfer the matrix to a disposable column.

Note: If maintenance of the sample in batch format is desired, do not transfer the matrix to the column. All centrifugations for washing and elution may be performed at 500 × g for 5 mins.

4. Tap the column to dislodge any trapped air bubbles in the matrix bed. Allow to settle.
5. Remove the bottom cap and save for later use. Allow the column to drain.

Note: Gentle pressure with a gloved thumb over the top of the column may be required to start the flow of liquid

Note: The majority of the flow-through can be discarded. However, a sample should be retained for analysis by SDS-PAGE or CDNB assay (see GST Detection Module, 27-4590-01) to measure the efficiency of binding to the matrix.

6. Wash the matrix with 10 bed volumes of binding buffer. Allow the column to drain. Repeat twice more for a total of three washes.

Note: GST-tagged protein bound to the matrix may be eluted directly (see below) at this stage using glutathione elution buffer (prepared as described in "Essential preliminaries") or the protein may be cleaved while bound to the matrix with the appropriate site-specific protease to liberate the protein of interest from the GST moiety.

Elution

7. Once the column with bound protein has been washed and drained, replace the bottom cap.

8. Elute the tagged protein by the addition of 1 ml of elution buffer (10 mM Glutathione in 50 mM Tris-HCl pH 8.0) per ml bed volume. Incubate the column at room temperature (22–25°C) for 10 min to elute the GST-tagged protein.

9. Remove the end cap and collect the eluate. This contains the GST-tagged protein.

10. Repeat the elution and collection steps twice. Pool the three eluates.

Note: Following the elution steps, a significant amount of GST-tagged protein may remain bound to the matrix. Volumes and times used for elution may vary among GST-tagged proteins. Additional elutions may be required. Eluates should be monitored for GST tagged protein by SDS-PAGE or by CDNB assay (GST Detection Module, 27-4590-01).

9. Appendix

9.1 Preparation of Buffers

Table 1 specifies volumes of supplied binding and elution buffer as well as the volume of distilled water required to prepare the buffers.

Table 1. Preparation of binding and elution buffer for different columns[1]

Column	Buffer	Phosphate buffer stock solution (ml)	Dilution buffer Tris-HCl (ml)	Glutathione (10 mM) (mg)	Dest. water	Final volume (ml)
GST SpinTrap (10×)	Binding	2.5	-	-	22.5	25
	Elution	-	5	15.4	-	5
GST GraviTrap	Binding	6	-	-	54	60
	Elution	-	10	30.8	-	10
GSTrap 1-ml	Binding	4.5	-	-	40.5	45
	Elution	-	10	30.8	-	10
GSTrap 5-ml	Binding	22.5	-	-	202.5	225
	Elution	-	50	154	-	50
GST MultiTrap (one 96 well plate)	Binding	43.2	-	-	388.8	432
	Elution	-	50	154	-	50

[1] GST Buffer Kit can also be used for batch/gravity flow purification using Glutathione Sepharose media packed in e.g. empty disposable PD-10 columns.

Note: Reducing agents, e. g. DTT, DTE, TCEP and β-mercaptoethanol, needs to be fresh. Add, therefore, the reducing agent to the sample and buffers just prior to equilibration of the wells.

9.2 Detection of GST-tagged proteins

Products provided of GE Healtchare
The GST Detection Modules (exists as both single and 96 well detection modules) provides convenient format for rapidly determination/screening of GST-tagged proteins. The module uses immobilized goat polyclonal Anti-GST Antibody to capture GST-tagged proteins from complex mixtures and exhibit very low, non-specific background binding. Using a chromogenic substrate, the system can detect as little as 1 ng of recombinant GST, providing a level of sensitivity that is 10 to 100 times greater than capture plates using immobilized glutathione. Anti-GST Antibody is also available as a stand alone product in 0.5 ml suitable for 50 detections.

CDNB assay
In addition to SDS-PAGE analysis of recombinants, the relative level of expression of GST-tagged protein can be estimated using the GST substrate CDNB (1-chloro-2,4-dinitrobenzene) which is included in the GST Detection Module.

Additional analyses with GST Detection Module
If recombinants expressing tagged proteins cannot be identified using the methods described above, clones can also be identified by ECL™ GST Western blotting Detection Kit for analysis using the Anti-GST Antibody contained in the GST Detection Module or available as a stand-alone product. Another alternative is to perform a functional assay, if available, specific for the protein of interest. The yield of GST- tagged protein can be estimated by measuring the absorbance at 280 nm. The amount of GST affinity tag can be approximated by 1 $A_{280} \approx 0.5$ mg/ml (This value is based on the extinction coefficient of the GST monomer using a Bradford protein assay. Other protein determination methods may produce different extinction coefficients). The yield of protein may also be determined by standard chromogenic methods (e.g. Lowry, BCA, Bradford, etc.). If

a Lowry or BCA type method is to be used, the sample must first be dialyzed against 2 000 volumes of 10 mM PBS to remove glutathione, which can interfere with protein measurement. The Bradford method can be performed in the presence of glutathione.

SDS-PAGE electrophoresis with Coomassie staining and if more sensitivity is needed Deep Purple™

1. Transfer 10 µl aliquots of each sample to be analyzed (e.g. samples retained following cell resuspension and lysis, column flow through, washes, eluates, etc.) to fresh tubes.
2. To each sample, add 30 µl SDS loading buffer (OBS! The volume depends on concentration of SDS in loading buffer). Vortex briefly and heat for 5 mins at 90–100°C.
3. Load the samples onto a 10–12.5% SDS-polyacrylamide gel.
4. Run the gel for the appropriate time and stain with Coomassie blue/deep purple according to existing protocols to visualize the parental GST (synthesized in control cells carrying the parental pGEX vector) and the tagged protein.

Note: Transformants expressing the desired tagged protein will be identified by the presence of a novel tagged protein larger than the M_r 29 000 size of parental GST*. If the above analysis indicates that the GST tagged protein has adsorbed to the Glutathione Sepharose 4B, you may proceed to large-scale purification. If the GST-tagged protein is absent from the purified material, it may be insoluble or expressed at very low levels; refer to the troubleshooting guide (page 21) for a discussion of this problem. Interpretation is sometimes complicated when GST-tagged proteins break down and release M_r 26 000 GST moiety. Such cases are usually recognized by the reduced level of the full size GST-tagged protein, and by the series of larger, partial proteolytic fragments down to M_r 26 000

* Parental pGEX vectors produce a M_r 29 000 GST –tagged protein containing amino acids coded for by the pGEX multiple cloning site.

Regeneration of Glutathione Sepharose 4B

1. Wash the gel with two bed volumes* of 0.1 M Tris HCl + 0.5 M NaCl, pH 8.5.
2. Wash the gel with two bed volumes of 0.1 M sodium acetate + 0.5 M NaCl, pH 4.5.
3. Repeat the above steps three to four times to give a total of four to five wash cycles of alternate buffers.
4. Re-equilibrate with 3–5 bed volumes of 1 × PBS. If the gel appears to be losing binding capacity, it may be due to an accumulation of precipitated, denatured or nonspecifically bound proteins. To remove precipitated or denatured substances, wash the matrix with 2 bed volumes of 6 M guanidine hydro chloride and then wash immediately with 5 bed volumes of 1 × PBS. To remove hydrophobically bound substances, wash the matrix with 3–4 bed volumes of 70% ethanol or with 2 bed volumes of a non-ionic detergent (conc. 0.1%), immediately followed by a wash with 5 bed volumes of 1 × PBS.

For long-term storage (> 1 month) the following procedure of additional washes is recommended:

5. Wash the gel twice with 10 bed volumes of 1 × PBS.
6. Repeat washes using 20% ethanol.
7. Store at 4–30°C
8. Re-equilibrate the gel with 1 × PBS before re-use.

* Bed volume is equal to 0.5 × the volume of the 50% Glutathione Sepharose slurry used or 0.75 × the volume of the original Glutathione Sepharose slurry. RediPack columns contain a 2 ml bed volume.

10. Troubleshooting

Consult the GST Gene Fusion System Handbook for more detailed information and references of the suggestion of solutions given below; see ordering information and pGEX instructions regarding troubleshooting recommendations for expression, fermentation, and solubilization.

Problem: No GST-tagged protein is detected by Coomassie-stained SDS gel of the bacterial sonicate.

Possible causes	*Solutions*
1. Optimize expression conditions.	Optimization of expression conditions can dramatically improve yields. Investigate the effects of cell strain, medium composition, incubation temperature and induction conditions on GST-tagged protein yield. Exact conditions will vary for each GST-tagged protein.
2. Check DNA sequences.	It is essential that protein-coding DNA sequences be cloned in the proper translation frame in pGEX vectors. Cloning junctions should be sequenced using 5′ pGEX sequencing primer and 3′ pGEX sequencing primer to verify that inserts are in-frame with GST. The reading frame of the multiple cloning site for each pGEX vector is shown GST Gene Fusion System Handbook
3. Analyze a small aliquot of an overnight culture by SDS-PAGE.	Generally, a highly expressed protein will be visible by Coomassie staining when 5–10 µl of an induced culture whose A_{600} is ~ 1.0 is loaded on the

Problem: No GST-tagged protein is detected by Coomassie-stained SDS gel of the bacterial sonicate. *continued.*

Possible causes	*Solutions*
3. Analyze a small aliquot of an overnight culture by SDS-PAGE. *continued.*	gel. Nontransformed host *E. coli* cells and cells transformed with the parental pGEX vector should be run in parallel as negative and positive controls, respectively. The presence of the GST-tagged protein in this total cell preparation and its absence from a clarified sonicate may indicate the presence of inclusion bodies (see below).
4. Check for expression by immunoblotting.	Some GST-tagged proteins may be masked on an SDS-polyacrylamide gel by a bacterial protein of approximately the same molecular weight. Immunoblotting can be used to identify GST-tagged proteins in these cases. Run an SDS-poly acrylamide gel of induced cells as above and transfer the proteins to a nitrocellulose or PVDF membrane. Detect GST-tagged protein using anti-GST antibody (included in the GST Detection Module).

Problem: Majority of GST-tagged protein is found in post-lysis pellet.

SDS-PAGE analysis of samples collected during the preparation of the bacterial lysis may indicate that the majority of the GST-tagged protein is located in the pellet. Possible causes and solutions are discussed below.

Possible causes	*Solutions*
1. Lysis may be insufficient.	Cell disruption is evidenced by partial clearing of the suspension or may be checked by microscopic examination. If the lysate is too viscous for handling, DNase I may be added to a final concentration of 10 µg/ml during lysozyme treatment.
2. GST-tagged protein may be insoluble (inclusion bodies).	If insufficient protein is found in the soluble fraction following centrifugation of the sonicate, it may be necessary to alter growth conditions: • GST-tagged protein solubility can be dramatically increased by lowering the growth temperature during induction. Experiment with growth temperatures in the range of 20–30°C. • Alter level of induction by decreasing IPTG concentration to < 0.1 mM. • Alter timing of induction. • Induce for a shorter period of time. • Induce at a higher cell density for a short period of time. • Increase aeration. High oxygen

Problem: Majority of GST-tagged protein is found in post-lysis pellet. *continued.*

Possible causes	*Solutions*
2. GST-tagged protein may be insoluble (inclusion bodies). *continued.*	transport can help prevent the formation of inclusion bodies. It may be necessary to combine the above approaches. Exact conditions must be determined empirically for each GST-tagged protein. If the above techniques do not significantly improve expression of soluble GST-tagged protein, protein can be solubilized from inclusion bodies using common denaturants such as 4–8 M guanidine hydrochloride, 4–8 M urea, detergents, alkaline pH (> 9), organic solvents, N-lauroyl-sarcosine. Other variables that affect solubilization include time, temperature, ionic strength, ratio of denaturant to protein and the presence of thiol reagents. Following solubilization, proteins must be properly refolded to regain function. Denaturant can be removed by dialysis, dilution, or gel filtration to allow refolding of the protein and formation of the correct intramolecular associations. Critical parameters during refolding include pH, presence of thiol reagents and the speed of denaturant removal. Once refolded, protein may be purified by ion exchange, gel filtration or affinity chromatography. GST-tagged proteins

Problem: Majority of GST-tagged protein is found in post-lysis pellet. *continued.*

Possible causes	*Solutions*
2. GST-tagged protein may be insoluble (inclusion bodies). *continued.*	can be purified to some extent while denatured. In some instances where GST-tagged proteins formed inclusion bodies, solubilization and binding to Glutathione Sepharose 4B was achieved in the presence of 2–3 M guanidine hydrochloride or urea. Success has also been achieved using up to 2% Tween 20 for solubilization and binding. Binding to Glutathione Sepharose 4B can also be achieved in the presence of 1% CTAB, 10 mM DTT or 0.03% SDS. Success of affinity purification in the presence of these agents will depend on the nature of the GST-tagged protein.

Problem: GST-tagged protein does not bind to Glutathione Sepharose 4B.

Possible causes	*Solutions*
1. Poor equilibration of Glutathione Sepharose 4B before use.	Check that the medium has been equilibrated with a buffer between pH 6.5 to 8.0 (e.g. PBS) before the cell lysate is applied. Binding of GST-tagged proteins to Glutathione Sepharose 4B is not efficient at pH less than 6.5 or greater than 8.
2. To short incubation time	GST-tagged proteins have a slow kinetic towards Glutathione Sepharose 4B and it may be necessary to increase the incubation time for the sample.

Problem: GST-tagged protein does not bind to Glutathione Sepharose 4B. *continued.*

Possible causes	*Solutions*
3. Low expressed protein.	The binding capacity is concentration dependent. Low expressed GST-tagged proteins therefore have general a poor binding affinity to Glutathione Sepharose 4B. Concentrate the sample before adding it to Glutathione Sepharose 4B slurry/column.
4. Test binding of GST from parental pGEX.	Prepare a lysate of cells harboring the parental pGEX plasmid and check binding to the matrix. If GST produced from the parental plasmid binds with high affinity, then the fusion partner may have altered the conformation of GST, thereby reducing its affinity. Adequate results may be obtained by reducing the temperature used for binding to 4°C, and by limiting the number of washes.
5. Masked binding site.	The addition of DTT to a final concentration of 5 mM prior to cell lysis can significantly increase binding of some GST tagged proteins to Glutathione Sepharose 4B.
6. Too extensive sonication/ mechanical lysis.	Too extensive sonication/mechanical lysis may denaturize the GST-tag which then prevents binding. Use as mild conditions as possible during cell lysis.

Problem: GST-tagged protein is not eluted from Glutathione Sepharose 4B.

Possible causes	*Solutions*
1. Insufficient elution.	Increase the duration of elution. In some instances, overnight elution at room temperature or 4°C is most effective.
2. Increase the volume of elution buffer.	Note that Glutathione Sepharose 4B will also function as a gel filtration medium with an approximate molecular weight exclusion limit of Mr 2 x 10^7. Small proteins (especially those liberated following cleavage with a site-specific protease) may require large elution volumes. Proteins eluted in a large volume may require concentration by ultra filtration.
3. Increase the concentration of glutathione in the elution buffer.	General protocols use 10 mM glutathione for elution which should be sufficient for most applications. Additional reduced glutathione must be obtained separately. Keep in mind that if the glutathione concentration is significantly increased above 15 mM, the buffer concentration will have to be increased to maintain proper pH.
4. Increase the ionic strength of the elution buffer.	The addition of 0.1–0.2 M NaCl to the elution buffer may also improve results. However, proteins that are very hydrophobic may precipitate in the presence of high salt concentrations;

Problem: GST-tagged protein is not eluted from Glutathione Sepharose 4B. *continued.*

Possible causes	*Solutions*
4. Increase the ionic strength of the elution buffer. *continued.*	here, addition of a non-ionic detergent may improve results (see below).
5. Add a non-ionic detergent to the elution buffer.	Nonspecific hydrophobic interactions may prevent solubilization and elution of GST- tagged proteins from Glutathione Sepharose 4B. Addition of a non-ionic detergent can improve results. The addition of 0.1% Triton X-100 or 2% N-octyl gluco side can significantly improve elution of some GST tagged proteins.

Problem: Insufficient purity of GST tagged protein

Possible causes	*Solutions*
1. Purification of a GST-tagged protein from *E. coli* sample which results in multiple bands after electrophoresis/ Western blotting analysis of eluted target protein	A M_r 70 000 protein is co-purifying with the GST-tagged protein The M_r 70 000 protein may be a protein product of the *E. coli* gene dnaK. This protein is involved in protein folding in *E. coli*. It has been reported that this association can be disrupted by incubating the tagged protein in 50 mM Tris-HCl, 2 mM ATP, 10 mM $MgSO_4$, pH 7.4 for 10 min. at 37°C prior to loading on GST SpinTrap. Alternatively, remove the DnaK protein by passing the tagged protein solution

Problem: Insufficient purity of GST tagged protein

Possible causes	*Solutions*
1. Purification of a GST-tagged protein from *E. coli* sample which results in multiple bands after electrophoresis/ Western blotting analysis of eluted target protein. *continued.*	through ATP-agarose or by ion exchange.
2. Partial degradation of tagged protein by proteases in lysate.	Add a protease inhibitor. Adding 1 mM PMSF or Pefabloc™ SC to the lysis solution may improve results. **Note:** Serine protease inhibitors must be removed prior to cleavage by thrombin or factor Xa. PreScission Protease is not a consensus serine protease and is insensitive to many of the protease inhibitors tested at GE Healthcare.
3. Host strain is not protease deficient.	Use a protease-deficient host. Multiple bands may be the result of proteolysis in the host bacteria. If this is the case, the use of a protease-deficient strain may be required (e.g. *lon*- or *ompT*). *E. coli* BL21 is available as a stand alone product. This strain is *ompT*

11. Ordering information

Product	Quantity	Code No
GST Bulk Kit	1	27-4570-01

Accessories	Quantity	Code No
LabMate™ PD-10 Buffer Reservoir	10	18-3216-03
GST Buffer Kit	1	28-9523-61
PD-10 Desalting Columns	30	17-0851-01

Related product	Quantity	Code No
GST GraviTrap™	10 × columns	28-9523-60
GSTrap™ 4B	5 × 1 ml	28-4017-45
GSTrap 4B	100 × 1 ml[1]	28-4017-46
GSTrap 4B	1 × 5 ml	28-4017-47
GSTrap 4B	5 × 5 ml	28-4017-48
GSTrap 4B	100 × 5 ml[1]	28-4017-49

[1] Pack size available by specific customer order.

Small scale product	Quantity	Code No
GST SpinTrap™	50 columns	28-9523-59
GST MultiTrap™ 4B	4 × 96-well plates	28-4055-00
GST MultiTrap 4 FF	4 × 96-well plates	28-4055-01

GST detection product	Quantity	Code No
GST Detection Module	50 detections	27-4590-01
GST Detection Module (96-well format)	5 × 96-well plates	27-4592-01
Anti-GST Antibody	0.5 ml, 50 detections	27-4577-01
ECL GST Western Blotting Detection Kit	1	RPN1237

GST cloning product	Quantity	Code No
pGEX 5′ Sequencing Primer 5′-d [GGG-CTGGCAAGCCACGTTTGGTG]-3′	0.05 A_{260} uni	27-1410-01
pGEX 3′ Sequencing Primer 5′-d [CCG-GGAGCTGCATGTGTCAGAGG]-3′	0.05 A_{260} unit	27-1411-01
E. coli BL21	1 vial	27-1542-01
13 different pGEX vectors	5 µg or 25 µg	**

** see www.gelifesciences.com

Site-specific Proteases	Quantity	Code No
PreScission™ Protease	500 units	27-0843-01
Thrombin	500 units	27-0846-01
Factor Xa	400 units	27-0849-01

Lysis kit	Quantity	Code No
Yeast Protein Extraction Buffer Kit	1	28-9440-45
Mammalian Protein Extraction Buffer	1	28-9412-79

Literature	Code No
GST Gene Fusion System Handbook	18-1157-58
Recombinant Protein Purification Handbook, Principles and methods	18-1142-75
Affinity Chromatography Handbook, Principles and methods	18-1022-29

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