Instructions 28407075 AD | **96-well plates**

GST MultiTrap™ FF and GST MultiTrap 4B

Contents

1 Introduction 3
2 Properties of 96-well filter plate and resins 4
3 General considerations 7
Recommended buffers 8
4 Sample preparation 9
Recommended four-step protocol for cell lysis 9
5 Purification protocols 10
Protein purification protocol using centrifugation 10
Protein purification protocol using vacuum 13
6 Troubleshooting 16
7 Ordering information 20
7 Appendix: Buffers 22

1 Introduction

GST MultiTrap FF and GST MultiTrap 4B are prepacked disposable 96-well filter plates for reproducible high-throughput screening of glutathione S-transferase (GST) tagged proteins. The 96-well filter plates are prepacked with Glutathione Sepharose™ 4 Fast Flow and Glutathione Sepharose 4B, respectively. GST-fusion proteins can be produced using the pGEX series of expression vectors.

These filter plates simplify the purification screening and small scale purification of up to 0.5 mg of GST-tagged proteins/well. After thorough cell disruption, it is possible to apply unclarified lysate directly to the wells in the 96-well filter plate without pre-centrifugation and/or filtration of the sample. It is recommended to extend the duration of mechanical/chemical lysis if the sample is too viscous after lysis. The tagged proteins are eluted under mild, nondenaturing conditions that preserve protein antigenicity and function.

Prepacked GST MultiTrap FF and GST MultiTrap 4B plates give high consistency in reproducibility well-to-well and plate-to-plate. The repeatability of yield and purity of eluted protein is high. Automated robotic systems as well as manual handling using centrifugation or vacuum pressure can be used. The purification protocol can easily be scaled up since Glutathione Sepharose is available in larger prepacked formats: GSTrap™ FF and GSTrap 4B (1-mL and 5-mL columns) and GSTPrep™ FF 16/10 (20-mL column). For order information see page 20.

Glutathione Sepharose 4 Fast Flow and Glutathione Sepharose 4B are compatible with a wide range of additives used in protein screening purification. The design of the 96-well filter plate in combination with the resin, provides fast, simple and convenient parallel purifications. A short purification time generally minimizes deleterious effects, such as degradation and oxidation of sensitive target proteins.

2 Properties of 96-well filter plate and resin

GST MultiTrap FF and GST MultiTrap 4B (Figure 1) are prepacked with the affinity resin Glutathione Sepharose 4 Fast Flow (4% highly cross-linked agarose beads) and Glutathione Sepharose 4B (4% agarose beads), respectively. Glutathione ligands are coupled via 10-carbon linkers to both matrices. The coupling is optimized to give high binding capacity (0.5 mg) for GST-tagged proteins and other gluthathione binding proteins. Note that the protein binding is protein dependent. The 96-well filter plates with 800 µL wells are made of polypropylene and polyethylene. In Table 1 characteristics of GST MultiTrap FF and GST MultiTrap 4B are presented. Every MultiTrap 96-well filter plate is labelled with a barcode for sample tracking, allowing efficient and reliable monitoring of the experiments.

Fig 1. GST MultiTrap FF and GST MultiTrap 4B 96-well filter plates

Table 1. GST MultiTrap FF and GST MultiTrap 4B characteristics

Filter plate size	127.8 × 85.5 × 30.6 mm according to ANSI/SBS[1] 1-2004, 3-2004 & 4-2004 standards
Filter plate material	Polypropylene and polyethylene
Resin	*GST MultiTrap FF:* Glutathione Sepharose 4 Fast Flow; Highly cross-linked spherical agarose, 4%, with a glutathione ligand coupled via a 10-carbon linker arm. *GST MultiTrap 4B:* Glutathione Sepharose 4B; 4% spherical agarose, with a glutathione ligand coupled via a 10-carbon linker arm.
Average bead size	*Glutathione Sepharose 4 Fast Flow:* 90 µm *GlutathioneSepharose 4B:* 90 µm
Binding capacity	*GST MultiTrap FF:* Up to 0.5 mg GST-tagged protein/well *GST MultiTrap 4B:* Up to 0.5 mg GST-tagged protein/well Binding capacity might differ depending on protein
Reproducibility between wells[2]	± 10%
Volume packed resin/well	50 µL (500 µL of 10% slurry)
Well volumes	800 µL
Number of wells	96
Centrifugation speed recommended maximum	Depends on sample pretreatment and sample properties. 100 to 500 × *g* 700 × *g*
Vacuum pressure recommended maximum	Depends on sample pretreatment and sample properties. - 0.1 to - 0.3 bar - 0.5 bar
Chemical stability	All commonly used aqueous buffers, e.g., 1 M acetate, pH 4.0 and 6 M guanidine hydrochloride for 1 hour at room temperature.
pH stability	Sepharose 4 Fast Flow pH 3 to 12 Sepharose 4B pH 4 to 13
Storage solution	20% ethanol
Storage temperature	2°C to 8°C

[1] ANSI = American National Standards Institute. SBS = Society for Biomolecular Screening.

[2] The amount of eluted target proteins/well does not differ more than ± 10% from the average amount/well for the whole filter plate.

The resin is compatible with all commonly used aqueous buffers, reducing agents, denaturants, such as 6 M guanidine-HCl (Gua-HCl) and 8 M urea, and a range of other additives (see Table 2).

Table 2. Glutathione Sepharose 4 Fast Flow or Glutathione Sepharose 4B are stable towards the following compounds at least at the concentrations given

Compound	Concentration
Reducing agents	5 mM DTE 20 mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 40 mM reduced glutathione
Denaturing agents[1]	8 M urea 6 M guanidine-HCl
Detergents	2% Tween™ 20 (nonionic) 2% NP-40 (nonionic) 2% cholate (anionic) 1% CHAPS (zwitterionic)
Other additives	20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl
Buffers	50 mM sodium phosphate, pH 7.4 100 mM Tris-HCl, pH 7.4 100 mM Tris-acetate, pH 7.4 100 mM HEPES, pH 7.4 100 mM MOPS, pH 7.4

[1] Denaturing agents are compatible with the resin. However, the GST-tag would be denatured using 6 M guanidine-HCl or 8 M urea. Lower concentrations can be used but have to be optimized before the run and the binding capacity might decrease.

3 General considerations

Unclarified cell lysate

GST MultiTrap FF and GST MultiTrap 4B are designed to allow parallel purification of GST-tagged proteins directly from unclarified cell lysates. Sample preparation is performed by mechanical and/or chemical lysis. No centrifugation or filtration is needed before loading the sample onto the 96-well filter plate. If the sample is too viscous, an extension of the duration of mechanical treatment of the sample to ensure a more complete lysis is recommended (keep the sample on ice to prevent overheating).
Lysis with commercial kits works but could sometimes give incomplete degradation of the cell paste which could result in problems when removing the sample from the wells. Therefore, if problems with draining the wells occur, add either chemicals (see section 4, Sample preparation) or, centrifuge/filtrate the sample before adding it to the wells.

Choice of resin

The optimal resin for your target protein is protein dependent. The recommendation is to test both resins if the yield is very important. Both GST MultiTrap FF and GST MultiTrap 4B binds up to 0.5 mg protein/well.

Buffers

Binding at neutral to slightly alkaline pH (pH 7 to 8) is recommended. Sodium phosphate buffers are often used. Including salt in the buffers and samples eliminates ion-exchange effects but can also have a marginal effect on the retention of target proteins.
Membrane proteins are usually purified in the presence of a detergent in the sample and buffers. Notice that the NaCl concentration might have to be optimized to avoid precipitation.

DTT

DTT can promote a higher binding of GST-tagged proteins and can be added to both binding and elution buffers. A final concentration of 1 to 20 mM DTT can be used. Add fresh DTT prior to cell lysis and to buffers.

Removal of GST-tags

If removal of the GST-tag (a naturally occurring M_r 26 000 protein) is required, the tagged protein can be digested with an appropriate site-specific protease while bound to GST MultiTrap FF or GST MultiTrap 4B or, alternatively, after elution. Cleavage on GST MultiTrap FF or GST MultiTrap 4B eliminates the extra step of separating the released protein from GST, because the GST-tag remains bound. The target protein is eluted using binding buffer. More information about tag cleavage is written in instructions for GSTrap FF and GSTrap 4B (for Product code, see Ordering information, page 20).

Manual/robotic handling

Whatever conditions are chosen, GST MultiTrap FF and GST MultiTrap 4B filter plates can be operated manually by centrifugation or vacuum or by using a robotic system.

Scaling up

Both Glutathione Sepharose 4 Fast Flow and Glutathione Sepharose 4B can be used for scale up in HiTrap™ 1-mL and 5-mL columns. Keeping the same conditions (e.g., Sepharose 4 Fast Flow or Sepharose 4B resin, DTT concentration etc.) provides highly consistent results and shortens the optimization time at scale up. If larger scale is needed, GSTPrep FF 16/10 column (20-mL) is available for Glutathione Sepharose 4 Fast Flow. Note that the binding capacity might be protein dependent.

Recommended buffers

Recommended binding and elution buffers are listed in Table 3.

Table 3. Recommended buffers

Binding buffer[1]	PBS, pH 7.4 (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
Elution buffer[1]	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

[1] 1 to 20 mM DTT can be included in the binding and elution buffers. See appendix for buffer recipes.

4 Sample preparation

For optimal growth and induction, refer to established protocols.

It is recommended that the samples are prepared according to standard protocols (see also below). After mechanical and/or chemical lysis the sample can be applied directly to the wells without clarification, excluding the centrifugation and/or filtration steps (this applies to both GST MultiTrap FF and GST MultiTrap 4B).

Recommended four-step protocol for cell lysis

The protocol below has been used successfully in our own laboratories for lysis of *E. coli*, but other established procedures can also work.

1 **Dilution of cell paste**
Add 5 to 10 mL of binding buffer for each gram of cell paste.

2 **Enzymatic lysis**
0.2 mg/mL lysozyme, 20 µg/mL DNAse, 1 mM $MgCl_2$, 1 mM Pefabloc™ SC or PMSF (final concentrations). Stir for 30 min at room temperature or + 4°C depending on the sensitivity of the target protein.

3 **Mechanical lysis**
Sonication on ice, approximately 6 min (frequency 50%),
or
homogenization with a French press or other homogenizer,
or
freeze/thaw, repeated at least five times.

Note: Mechanical lysis time might have to be extended compared with standard protocols to secure an optimized lysate for sample loading (to prevent clogging of the wells). Different proteins have different sensitivity to cell lysis and caution has to be taken to avoid frothing and overheating of the sample.

4 **Adjustment of the pH of the lysate**
Measure and adjust pH if needed. Do not use strong bases or acids for pH-adjustment (due to precipitation risk). Apply the unclarified lysate to the wells in the 96-well filter plate **directly** after preparation.

Note: Unclarified cell lysate can precipitate unless used immediately or frozen before use. New lysis of the sample can then prevent clogging of the wells when loading the 96-well filter plate.

5 Purification protocols

Protein purification protocol using centrifugation

General considerations

- This protocol is a general guideline for the purification with GST MultiTrap FF and GST MultiTrap 4B. Optimization might be required depending on source and type of protein.
- Each well of the prepacked GST MultiTrap FF and GST MultiTrap 4B has a capacity of up to 0.5 mg of GST-tagged protein.
- Mix briefly before centrifugation in the equilibration, wash and elution steps to increase the efficiency of the step.
- If low yield of eluted target protein is obtained, the incubation time should be increased.
- In order to increase the purity and yield, DTT can be used in the sample and binding buffer.
- Do not apply more than 700 × *g* during centrifugation.
- Each well contains 500 µL 10% slurry of Glutathione Sepharose 4 Fast Flow or Glutathione Sepharose 4B in storage solution (50 µL resin in 20% ethanol).

See also Figure 2.

1 Peel off the bottom seal.

   Note: Hold the filter plate above a sink due to risk of small leakage of storage solution when removing the bottom seal.

2 Gently shake the 96-well filter plate while holding it upside down, to remove any resin stuck on the top seal. Place the filter plate in upright position.

3 Peel off the top seal from the filter plate while holding it against the bench surface.

   Note: If resin in one or several wells has dried out, add buffer to the wells to rehydrate the resin. The performance of the resin is not affected.

4 Position the filter plate on top of a collection plate.

   Note: Remember to change or empty the collection plate when necessary during the following steps.

5 Centrifuge the filter plates for 2 min at 500 × *g*, to remove the storage solution from the resin.

6. Add 500 µL deionized water/well.
   Centrifuge for 2 min at 500 × *g*.

7. Add 500 µL binding buffer/well, mix briefly to equilibrate the resin.
   Centrifuge for 2 min at 500 × *g*.
   Repeat once.

8. Apply unclarified or clarified lysate (maximum 600 µL/well) to the wells, mix briefly and incubate for 3 min.
   (Increase the incubation time if the yield is too low).

9. Remove the flow through by centrifuging for 4 min at 100 × *g* (or until all wells are empty).

10. Add 500 µL binding buffer/well, mix briefly to wash out unbound sample.
    Centrifuge at 500 × *g* for 2 min.
    Repeat once (or until all unbound sample are removed, A_{280} should be < 0.1 for high purity).

11. Add 200 µL* of elution buffer/well and mix for 1 min.
    Change collection plate and centrifuge the plates at 500 × *g* for 2 min and collect the fractions.
    Repeat twice (or until all target protein has been removed, A_{280} < 0.1 for high purity).
    If required, change collection plate between each elution (to prevent unnecessary dilution of the target protein).

    * The volumes can be varied depending on which concentration of target protein needed, for example, 50 or 100 µL elution buffer/well.

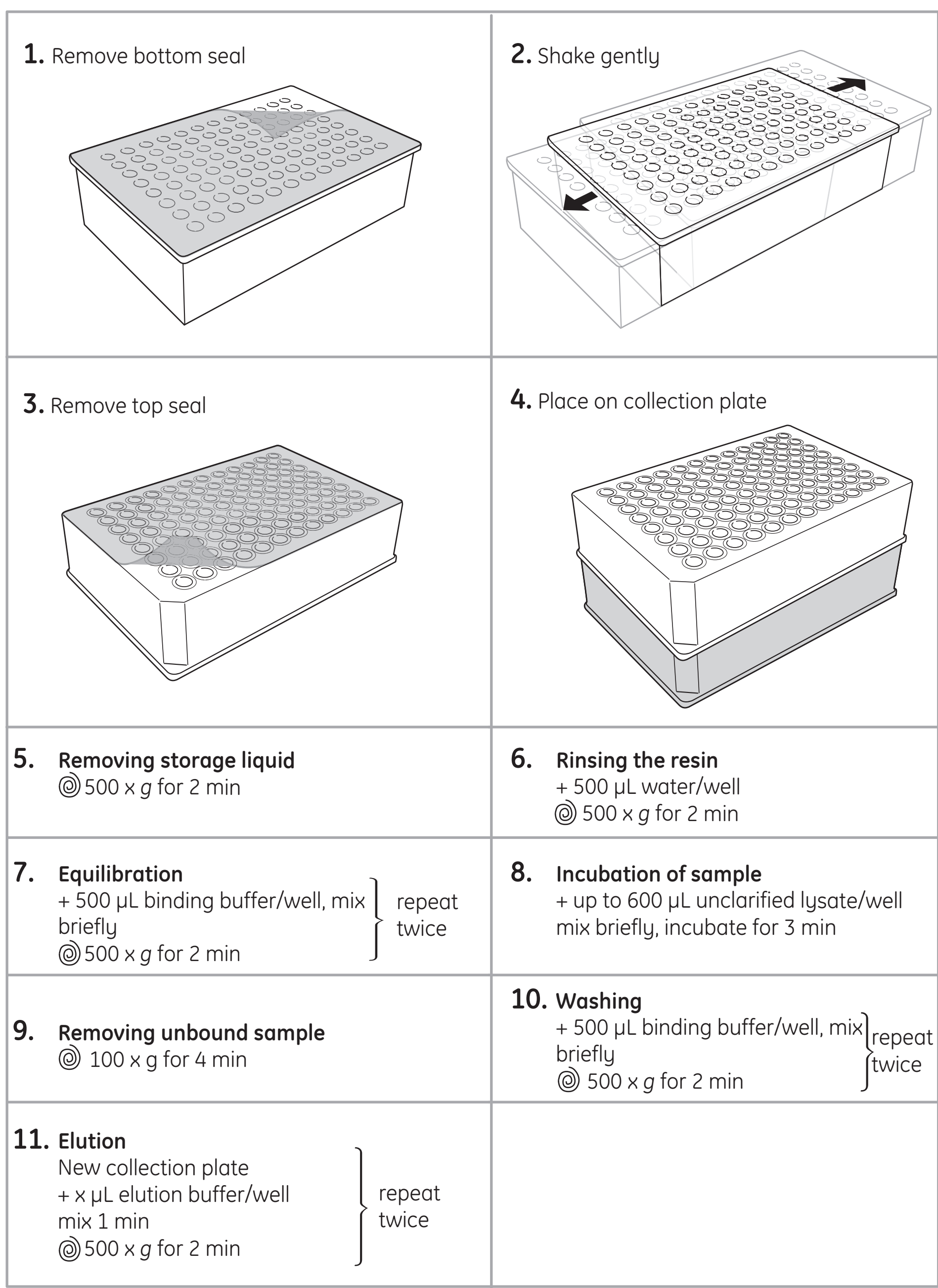


Fig 2. Purification work flow when using centrifugation (for details and tips, see page 10-11)

Protein purification protocol using vacuum

General considerations

- This protocol is a general guideline for the purification with GST MultiTrap FF and GST MultiTrap 4B. Optimization might be required depending on source and type of protein.
- Each well of the prepacked GST MultiTrap FF and GST MultiTrap 4B has a capacity of up to 0.5 mg of GST-tagged protein.
- Mix briefly before vacuum pressure in the equilibration, wash and elution steps to increase the efficiency of the step.
- If low yield of eluted target protein is obtained, the incubation time should be increased.
- In order to increase the purity and yield, DTT can be used in the sample and binding buffer.
- Do not apply more vacuum than - 0.5 bar.
- If problems with foaming, reproducibility or bubbles in the collection plate occur using vacuum, the centrifugation protocol should be considered.
- Each well contains 500 µL 10% slurry of Glutathione Sepharose 4 Fast Flow or Glutathione Sepharose 4B in storage solution (50 µL resin n 20% ethanol).

See also Figure 3.

1 Peel off the bottom seal.

   Note: Hold the filter plate above a sink due to risk of small leakage of storage solution when removing the bottom seal.

2 Gently shake the 96-well filter plate while holding it upside down, to remove any resin stuck on the top seal. Place the filter plate in up-right position.

3 Peel off the top seal from the filter plate while holding it against the bench surface.

   Note: If resin in one or several wells has dried out, add buffer to the wells to rehydrate the resin. The performance of the resin is not affected.

4 Position the filter plate on top of a collection plate.

   Note: Remember to change or empty the collection plate when necessary during the following steps.

5 Set the vacuum to - 0.15 bar.
Place the 96-well filter plate and collection plate on the vacuum manifold to remove the ethanol from the resin.

6 Add 500 µL deionized water/well.
Apply vacuum to drain the water from the wells.

7 Add 500 µL binding buffer/well, mix briefly to equilibrate the resin.
Remove the solution as in step 5.
Repeat once.

8 Apply unclarified or clarified lysate (maximum 600 µL/well) to the wells, mix briefly and incubate for 3 min.
(Increase the incubation time if the yield is too low)

Note: In purifications using a robotic system, the vacuum has to be adjusted to methods applicable to the system.

9 Remove the flow through by applying a vacuum of - 0.15 bar until all wells are empty.
Then *slowly* increase the vacuum to - 0.30 bar and turn off the vacuum after approximately 5 seconds.

Note: Increasing the vacuum too fast can give foam under the filter plate and cross-contamination can occur.

10 Add 500 µL binding buffer/well, mix briefly to wash out unbound sample.
Remove the solution as in step 9.
Repeat once (or until all unbound sample are removed, A_{280} should be < 0.1 for high purity).

11 Add 200 µL elution buffer* and mix for 1 min.
Change collection plate and elute the sample using vacuum, see step 9.
Repeat twice (or until all target protein has been removed, A_{280} < 0.1 for high purity).
If required, change collection plate between each elution (to prevent unnecessary dilution of the target protein).

* The volumes can be varied depending on which concentration of target protein needed, for example 50 or 100 µL elution buffer/well.

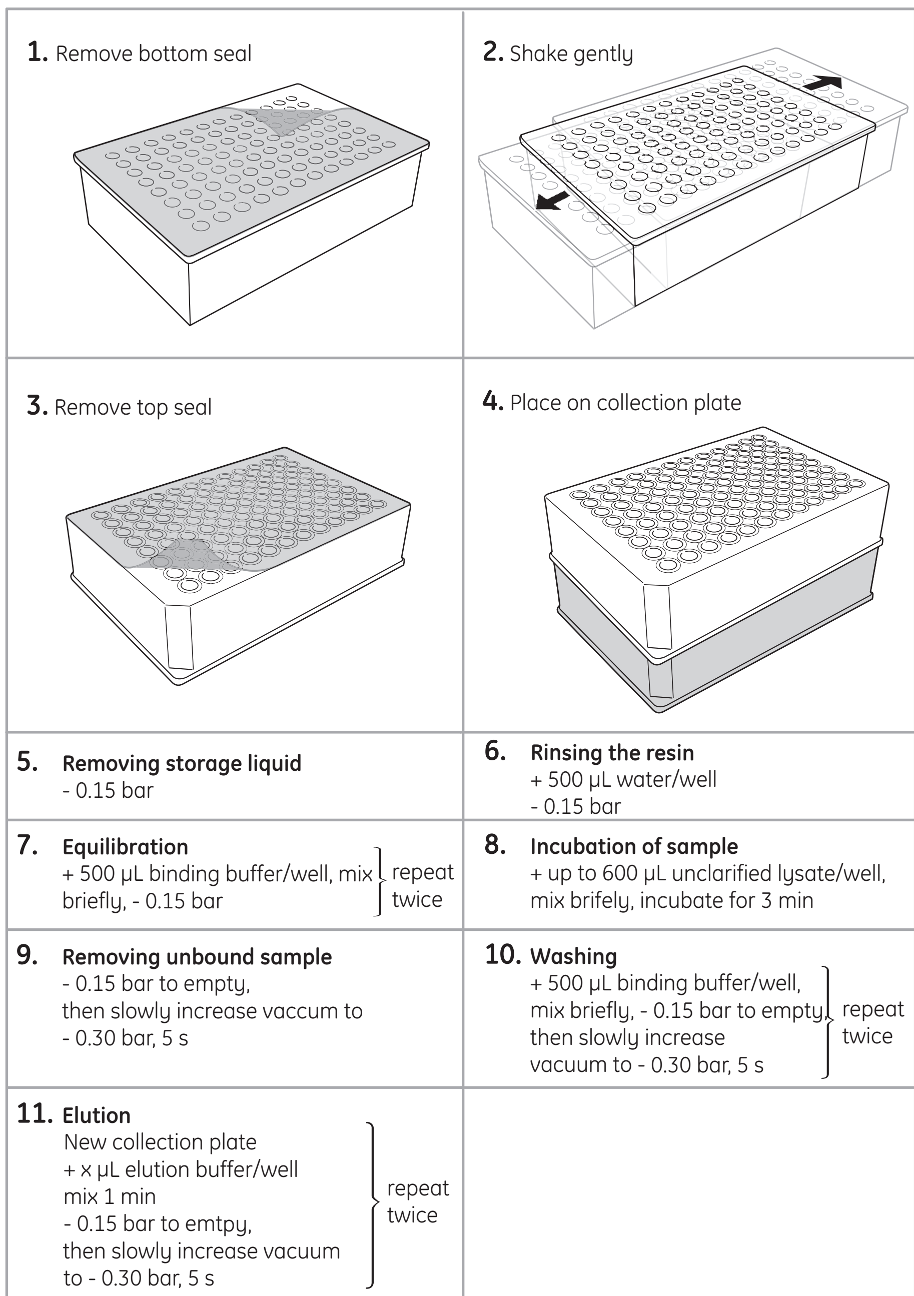


Fig 3. Purification work flow when using vacuum pressure (for details and tips, see page 13-14)

6 Troubleshooting

The following tips might be of assistance.
If you have any further questions about your GST MultiTrap FF or GST MultiTrap 4B 96-well filter plates, visit www.gehealthcare.com/protein-purification-labresearch, contact our technical support, or your local GE Healthcare representative.

Consult the GST Gene Fusion System Handbook (Product code 18115758) for more detailed information and pGEX instructions regarding troubleshooting recommendations for expression, fermentation and solubilization.

Fault	Possible cause	Action
GST-tagged protein does not bind	GST-tagged protein denatured by mechanical lysis. Too extensive lysis can denature the tagged protein and prevent it to bind.	• Use mild mechanical/chemical lysis conditions during cell lysis. Conditions for lysis must be empirically determined.
	Aggregation of GST-tagged proteins in sample which cause precipitation.	• Add DTT to the sample prior to cell lysis and also add DTT to the buffers. Adding DTT to a final concentration of 1 to 20 mM can significantly increase binding of some GST-tagged proteins.
	The fusion protein might have altered the conformation of GST, thereby reducing the affinity for the GST-tagged protein.	• Test the binding of GST from parental pGEX: Prepare a sonicate of cells harboring the parental pGEX plasmid and check binding to the matrix. If GST produced from the parental plasmid binds with high affinity, the fusion protein might have altered the conformation of GST, thereby reducing the affinity for the GST-tagged protein. • Adequate results can be obtained by reducing the temperature used for binding to + 4°C, and by limiting well washing.
	Equilibration is too short. Binding of GST-tagged proteins is not efficient at pH less than 6.5 or greater than 8.	• Equilibrate with a buffer pH 6.5 to 8.0 (e.g. PBS) before the clarified cell lysate is applied.
GST-tagged protein is not eluted efficiently.	—	• Increase the volume used for elution: Decrease the centrifugation speed during elution.

Fault	Possible cause	Action
	—	• Increase the concentration of glutathione in the elution buffer: The 10 mM recommended in this protocol should be sufficient for most applications, but exceptions exist. Try 50 mM Tris-HCl, 20 to 40 mM reduced glutathione, pH 8.0 as elution buffer.
	A low pH canlimit elution.	• Increase the pH of the elution buffer: Increasing the pH of the elution buffer to pH 8 to 9 might improve elution without requiring an increase in the concentration of glutathione used for elution. • Increase the ionic strength of the elution buffer: Adding 0.1 to 0.2 M NaCl to the elution buffer might also improve results.
	The glutathione in the elution buffer is oxidized.	• Use fresh elution buffer. • Add DTT.
	Non-specific hydrophobic interactions might prevent solubilization and elution of tagged proteins.	• Add a nonionic detergent to the elution buffer: Adding a nonionic detergent can improve results. Adding 2% N-octylglucoside can significantly improve elution of some GST-tagged proteins.
Multiple bands are observed after electrophoresis/ Western blotting analysis of eluted target protein.	M_r 70 000 protein co-purifies with the GST-tagged protein	• The M_r 70 000 protein is probably 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 filter plate. • Alternatively, remove the DnaK protein by passing the tagged protein solution through ATP-agarose or by ion exchange.

Fault	Possible cause	Action
	Partial degradation of tagged proteins by proteases	• Add a protease inhibitor: Multiple bands might be a result of partial degradation of tagged proteins by proteases. Adding 1 mM PMSF to the lysis solution can improve results. A non-toxic, water-soluble alternative to PMSF is 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (AEBSF), commercially available as Pefabloc SC from Boehringer Mannheim. **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.*
	Proteolysis in the host bacteria	• Use a protease-deficient host: Multiple bands might be the result of proteolysis in the host bacteria. If this is the case, the use of a host-deficient strain might be required (e.g. lon- or ompT). *E. coli* BL21 is provided with the pGEX vectors. This strain is ompT.
	Cell disruption during mechanical lysis	• Decrease lysis time: Cell disruption is apparent by partial clearing of the suspension and can be checked by microscopic examination. Adding lysozyme (0.1 volume of a 10 mg/mL lysozyme solution in 25 mM Tris-HCl, pH 8.0) prior to mechanical lysis can improve results. Avoid frothing as this might denature the fusion protein. Over-lysis can also lead to the co-purification of host proteins with the GST-tagged protein.
	Co-purification of a variety of proteins known as chaperonins	• Include an additional purification step: Additional bands might be caused by the co-purification of a variety of proteins known as chaperonins, which are involved in the correct folding of nascent proteins in *E. coli*. These include, but are not limited to: DnaK (M_r ~ 70 000), DnaJ (M_r ~ 37 000), GrpE (M_r ~ 40 000), GroEL (M_r ~ 57 000) and GroES (M_r ~10 000). Several methods for purifying GST-tagged proteins from these co-purifying proteins have been described.

Fault	Possible cause	Action
	Antibodies that react with various *E. coli* proteins might be present in your tagged protein sample	• Cross-adsorb antibody with *E. coli* proteins: Depending on the source of the anti-GST antibody, it might contain antibodies that react with various *E. coli* proteins that might be present in your tagged protein sample. Cross-adsorb the antibody with an *E. coli* sonicate to remove anti-*E. coli* antibodies from the preparation. Anti-GST antibody from GE Healthcare has been cross-adsorbed against *E. coli* proteins and tested for its lack of non-specific background binding in Western Blots.
Multiple bands are observed after electrophoresis analysis of cleaved target protein:	Proteolysis in the host bacteria	• Determine when the bands appear: Test to be certain that additional bands are not present prior to PreScission Protease, thrombin or factor Xa cleavage. Such bands might be the result of proteolysis in the host bacteria. • Tagged partner might contain recognition sequences for PreScission Protease, thrombin or factor Xa: Check the sequences. See the GST Gene Fusion System Handbook (Product code 18115758) for details.

7 Ordering information

Products	Quantity	Product code
GST MultiTrap FF	4 × prepacked 96-well filter plates	28405501
GST MultiTrap 4B	4 × prepacked 96-well filter plates	28405500

Related products	Quantity	Product code
GSTrap FF	2 × 1 mL	17513002
GSTrap FF	5 × 1 mL	17513001
GSTrap FF	100 × 1 mL*	17513005
GSTrap FF	1 × 5 mL	17513101
GSTrap FF	5 × 5 mL	17513102
GSTrap FF	100 × 5 mL*	17513105
GSTrap 4B	5 × 1 mL	28401745
GSTrap 4B	100 × 1 mL*	28401746
GSTrap 4B	1 × 5 mL	28401747
GSTrap 4B	5 × 5 mL	28401748
GSTrap 4B	100 × 5 mL*	28401749
GSTPrep FF 16/10	1 × 20 mL	17523401

* Pack size available by special order.

Accessories	Quantity	Product code
Collection Plate, 96-well plate 500 µl, V-shaped bottom	5 pack	28403943

Site-specific proteases	Quantity	Product code
PreScission Protease	500 units	27084301
Thrombin protease	500 units	27084601
Factor Xa protease	400 units	27084901

Companion products	Quantity	Product code
GST 96-well Detection Module	5 × 96 reactions	27459201
Anti-GST Antibody (50 detections)	0.5 mL	27457701

Literature	Product code
Recombinant Protein Handbook, Protein Amplification and Simple Purification	18114275
GST Gene Fusion System Handbook	18115758
Affinity Chromatography Handbook, Principle and Methods	18102229
Affinity Chromatography Columns and Resin Product Profile	18112186
GSTrap FF, Instruction*	71501696
GSTrap 4B, Instruction*	28404813

* Available on www.gehealthcare.com/protein-purification-labresearch

Appendix: Buffers

Binding and wash buffer: Phosphate buffer

Prepare 1 liter of 10 mM sodium phosphate, 140 mM NaCl, pH 7.4:

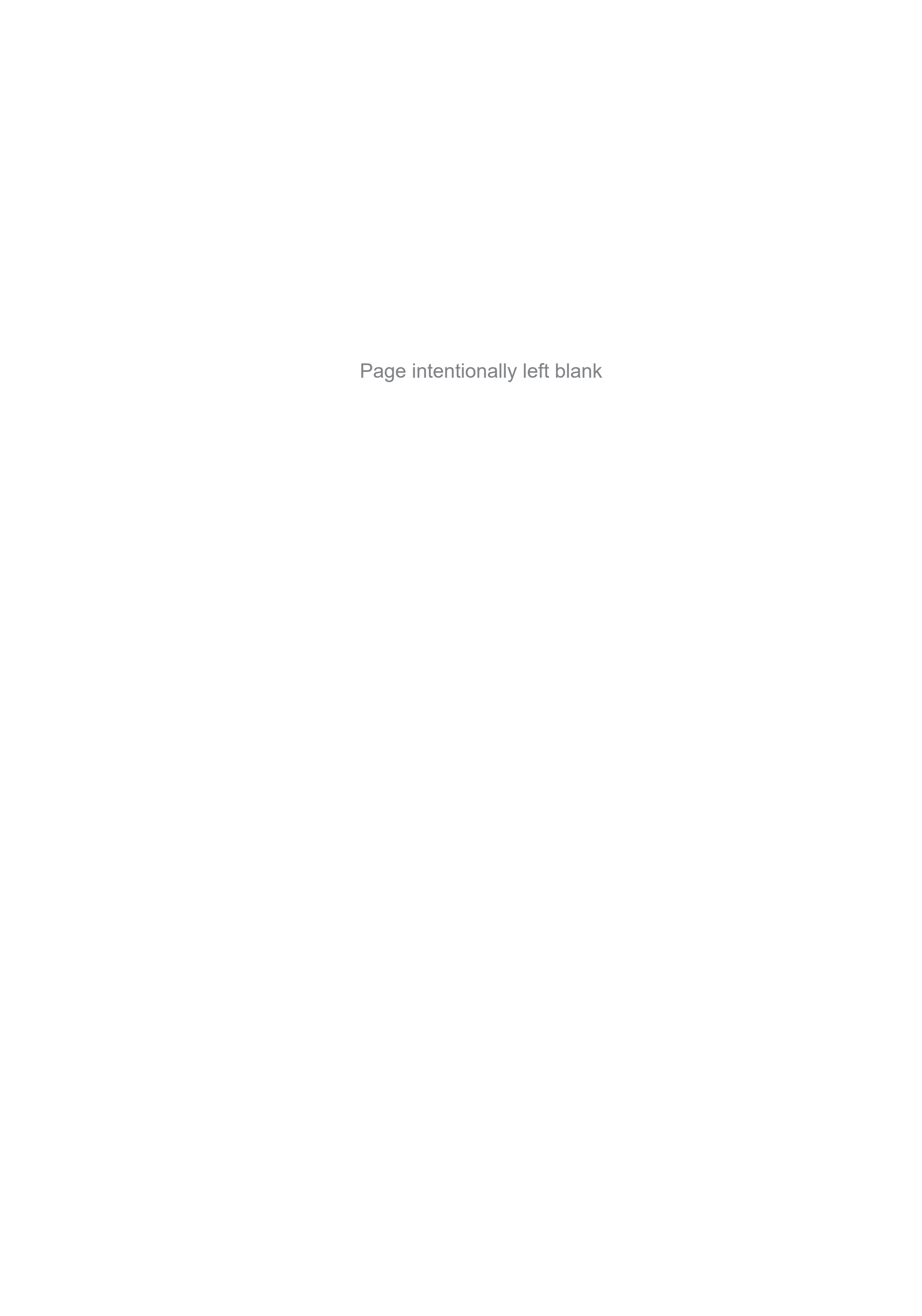
1. Add the following into a calibrated bottle:
   - 0.89 g $Na_2HPO_4 \times 2H_2O$ (177.99 g/mol)
   - 0.69 g $NaH_2PO_4 \times H_2O$ (137.99 g/mol)
   - 8.18 g NaCl (58.44 g/mol)
2. Add distilled water to 900 mL and dissolve completely.
3. Adjust pH from basic to 7.4 with HCl.
4. Add distilled water to 1000 mL and filter through a 0.45 µm filter.

Elution buffer: Tris-HCl including glutathione

Prepare 0.5 liter of 50 mM Tris-HCl, 10 mM glutathione, pH 8.0:

1. Add the following into a calibrated bottle:
   - 3.03 g Tris(hydroxymethyl)-aminomethan (121.14 g/mol)
   - 1.54 g Reduced glutathione (307.30 g/mol)
2. Add distilled water to 400 mL and dissolve completely.
3. Adjust pH from basic to 8.0 with HCl.
4. Add distilled water to 500 mL and filter through a 0.45 µm filter.
5. If needed, add a reducing agent (1 to 20 mM, depending on sample).

   Note: Reducing agents, for example, 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.


Page intentionally left blank

For contact information for your local office, visit:
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/sampleprep

GE Healthcare Europe GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare
100 Results way
Marlborough, MA 01752
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg.
3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

GE, the GE Monogram, GSTPrep, GSTrap, HiTrap, MultiTrap, PreScission, and Sepharose are trademarks of General Electric Company.

Pefabloc is a trademark of DSM IP Assets B.V. Tween is a trademark of the Croda Group of Companies.

All other third party trademarks are the property of their respective owners.

Licensing information
A license for commercial use of GST gene fusion vectors must be obtained from Chemicon International, Incorporated, 28820 Single Oak Drive, Temecula, California 92590, USA.

© 2006-2018 General Electric Company

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

28407075 AD 10/2018