GE Healthcare

Instructions 28-4070-75 AC **96-well plates**

GST MultiTrap FF and GST MultiTrap 4B

Within the United States of America [USA] and the Territories of the USA, this product is *To Be Used Solely for Research And Development Purposes Under the Direct Supervision of a Technically Qualified Individual.*

MultiTrap

Contents

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 may 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, non-denaturing 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 media, 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 media

GST MultiTrap FF and GST MultiTrap 4B (Fig. 1) are prepacked with the affinity media 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
Media	*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 may differ depending on protein
Reproducibility between wells[2]	±10%
Volume packed medium/well	50 µl (500 µl of 10% slurry)
Well volumes	800 µl
Number of wells	96
Centrifugation speed recommended maximum	Depends on sample pre-treatment and sample properties. 100 to 500 × *g* 700 × *g*
Vacuum pressure recommended maximum	Depends on sample pre-treatment 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 3–12 Sepharose 4B 4-13
Storage solution	20% ethanol
Storage temperature	+4 to 30°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 medium 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% Triton™ X-100 (nonionic) 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 media. However, the GST-tag would be denatured using 6 M guanidine-HCl or 8 M urea. Lower concentrations may be used but have to be optimized before the run and the binding capacity may 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 medium

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

Buffers

We recommend binding at neutral to slightly alkaline pH (pH 7–8). 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 may 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 1-20 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 Code No., 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 medium, 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 may 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] 1-20 mM DTT can be included in the binding and elution buffers. See appendix for buffer recipes.

4 Sample preparation

For optimal growth and induction, please 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 may also work.

1 **Dilution of cell paste**
Add 5–10 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 minutes at room temperature or +4 °C depending on the sensitivity of the target protein.

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

Note: Mechanical lysis time may 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 may 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 may 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 medium in 20% ethanol).

See also Fig. 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 medium 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 media in one or several wells has dried out, add buffer to the wells to rehydrate the media. The performance of the media 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 minutes at 500 × *g*, to remove the storage solution from the medium.

6 Add 500 µl deionized water/well.
Centrifuge for 2 minutes at 500 × *g*.

7 Add 500 µl binding buffer/well, mix briefly to equilibrate the medium.
Centrifuge for 2 minutes at 500 × *g*.
Repeat once.

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

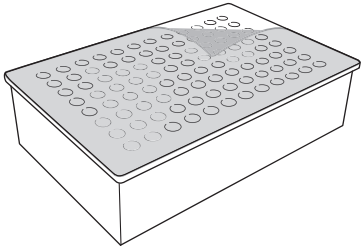
9 Remove the flow through by centrifuging for 4 minutes at 100 × *g* (or until all wells are empty).

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

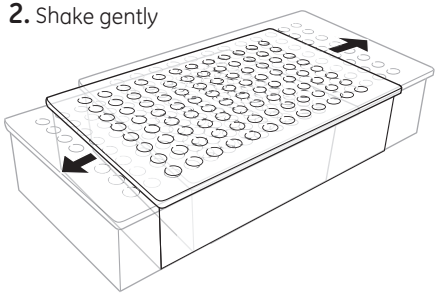
11 Add 200 µl* of elution buffer/well and mix for 1 minute.
Change collection plate and centrifuge the plates at 500 × *g* for 2 minutes and collect the fractions.
Repeat twice (or until all target protein has been removed, A_{280} < 0.1 for high purity).
If required, change collection plate between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

* The volumes can be varied depending on which concentration of target protein needed, *e.g.* 50 or 100 µl elution buffer/well.)

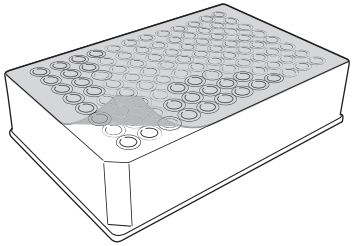
1. Remove bottom seal



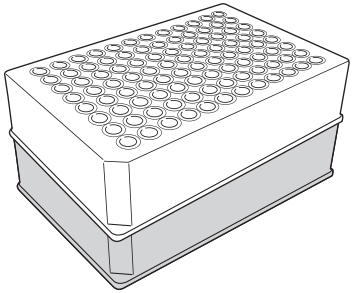
2. Shake gently



3. Remove top seal



4. Place on collection plate



5. **Removing storage liquid**
500 x *g* for 2 minutes

6. **Rinsing the medium**
+ 500 µl water/well
500 x *g* for 2 minutes

7. **Equilibration**
+ 500 µl binding buffer/well, mix briefly 500 x *g* for 2 minutes } repeat twice

8. **Incubation of sample**
+ up to 600 µl unclarified lysate/well
mix briefly, incubate for 3 minutes

9. **Removing unbound sample**
100 x *g* for 4 minutes

10. **Washing**
+ 500 µl binding buffer/well, mix briefly 500 x *g* for 2 minutes } repeat twice

11. **Elution**
New collection plate
+ x µl elution buffer/well
mix 1 minute
500 x *g* for 2 minutes } repeat twice

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 may 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 medium in 20% ethanol).

See also Fig. 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 medium 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 media in one or several wells has dried out, add buffer to the wells to rehydrate the media. The performance of the media 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 medium.

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 medium.
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 minutes.
(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.

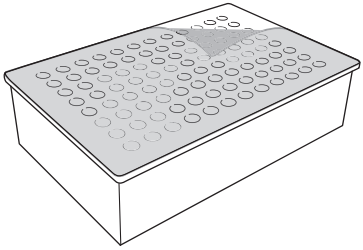
Note: Increasing the vacuum too fast can give foam under the filter plate and cross-contamination can occur.

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).

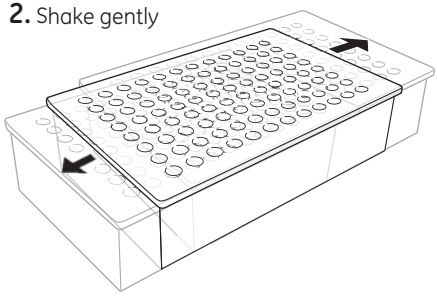
11 Add 200 µl elution buffer* and mix for 1 minute.
Change collection plate and elute the sample using vacuum, see step 9.
Repeat twice (or until all target protein has been removed, A_{280} < 0.1 for high purity).
If required, change collection plate between each elution (to prevent unnecessary dilution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

* The volumes can be varied depending on which concentration of target protein needed, *e.g.* 50 or 100 µl elution buffer/well.

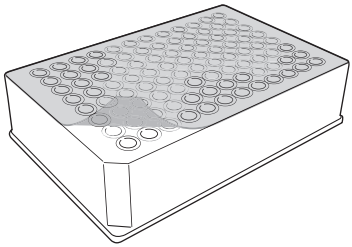
1. Remove bottom seal



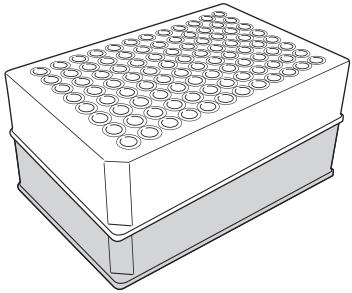
2. Shake gently



3. Remove top seal



4. Place on collection plate



5. **Removing storage liquid**
-0.15 bar

6. **Rinsing the medium**
+ 500 µl water/well
-0.15 bar

7. **Equilibration**
+ 500 µl binding buffer/well, mix briefly, -0.15 bar } repeat twice

8. **Incubation of sample**
+ up to 600 µl unclarified lysate/well, mix brifely, incubate for 3 minutes

9. **Removing unbound sample**
-0.15 bar to emtpy,
then slowly increase vaccum to -0.30 bar, 5 sec

10. **Washing**
+ 500 µl binding buffer/well,
mix briefly, -0.15 bar to empty,
then slowly increase vacuum to -0.30 bar, 5 sec } repeat twice

11. **Elution**
New collection plate
+ x µl elution buffer/well
mix 1 minute
-0.15 bar to emtpy,
then slowly increase vacuum to -0.30 bar, 5 sec } repeat twice

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

6 Troubleshooting

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

Consult the GST Gene Fusion System Handbook (Art.no. 18-1157-58) 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 1–20 mM may significantly increase binding of some GST-tagged proteins.
	The fusion protein may 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 may have altered the conformation of GST, thereby reducing the affinity for the GST-tagged protein. • Adequate results may 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, 20–40 mM reduced glutathione, pH 8.0 as elution buffer.
	A low pH may limit elution.	• Increase the pH of the elution buffer: Increasing the pH of the elution buffer to pH 8–9 may improve elution without requiring an increase in the concentration of glutathione used for elution. • Increase the ionic strength of the elution buffer: Adding 0.1–0.2 M NaCl to the elution buffer may also improve results.
	The glutathione in the elution buffer is oxidized.	• Use fresh elution buffer. • Add DTT.
	Non-specific hydrophobic interactions may prevent solubilization and elution of tagged proteins.	• Add a non-ionic detergent to the elution buffer: Adding a non-ionic detergent may improve results. Adding 0.1% Triton X-100 or 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 may be a result of partial degradation of tagged proteins by proteases. Adding 1 mM PMSF to the lysis solution may 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 may be the result of proteolysis in the host bacteria. If this is the case, the use of a host-deficient strain may 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 may improve results. Avoid frothing as this may 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 may 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 may not be 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 may be present in your tagged protein sample	• Cross-adsorb antibody with *E. coli* proteins: Depending on the source of the anti-GST antibody, it may contain antibodies that react with various *E. coli* proteins that may 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 may be the result of proteolysis in the host bacteria. • Tagged partner may contain recognition sequences for PreScission Protease, thrombin or factor Xa: Check the sequences. See the GST Gene Fusion System Handbook (Art.no. 18-1157-58) for details.

7 Ordering information

Products	No. supplied	Code no.
GST MultiTrap FF	4 × prepacked 96-well filter plates	28-4055-01
GST MultiTrap 4B	4 × prepacked 96-well filter plates	28-4055-00

Related products	No. supplied	Code no.
GSTrap FF	2 × 1 ml	17-5130-02
GSTrap FF	5 × 1 ml	17-5130-01
GSTrap FF	100 × 1 ml*	17-5130-05
GSTrap FF	1 × 5 ml	17-5131-01
GSTrap FF	5 × 5 ml	17-5131-02
GSTrap FF	100 × 5 ml*	17-5131-05
GSTrap 4B	5 × 1 ml	28-4017-45
GSTrap 4B	100 × 1 ml*	28-4017-46
GSTrap 4B	1 × 5 ml	28-4017-47
GSTrap 4B	5 × 5 ml	28-4017-48
GSTrap 4B	100 × 5 ml*	28-4017-49
GSTPrep FF 16/10	1 × 20 ml	17-5234-01

* Pack size available by special order.

Accessories	No. supplied	Code no.
Collection Plate, 96-well plate 500 µl, V-shaped bottom	5 pack	28-4039-43

Site-specific proteases	No. supplied	Code no.
PreScission Protease	500 units	27-0843-01
Thrombin protease	500 units	27-0846-01
Factor Xa protease	400 units	27-0849-01

Companion products	No. supplied	Code no.
GST 96-well Detection Module	5 × 96 reactions	27-4592-01
Anti-GST Antibody (50 detections)	0.5 ml	27-4577-01

Literature	Code no.
Recombinant Protein Handbook, Protein Amplification and Simple Purification	18-1142-75
GST Gene Fusion System Handbook	18-1157-58
Affinity Chromatography Handbook, Principle and Methods	18-1022-29
Affinity Chromatography Columns and Media Product Profile	18-1121-86
GSTrap FF, Instruction*	71-5016-96
GSTrap 4B, Instruction*	28-4048-13

* 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 (1-20 mM, depending on sample).

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.

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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.

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