Instructions 28-4048-13 AF

GSTrap™ 4B, 1 ml and 5 ml

GSTrap 4B columns are prepacked 1 ml and 5 ml HiTrap™ columns for convenient, one-step purification of glutathione S-transferase (GST) tagged proteins produced using the pGEX series of expression vectors, other glutathione S-transferases, and glutathione binding proteins.

GST-tagged proteins can be purified directly from pretreated bacterial lysates using GSTrap 4B. Tagged proteins are eluted under mild, non-denaturing conditions that preserve protein antigenicity and function.

The matrix, Glutathione Sepharose™ 4B, is also available as lab packages and is a good choice for scale-up.

The columns can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™.

Table of contents

Please read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

1 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

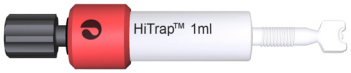

Fig 1. HiTrap, 1 ml column.

Fig 2. HiTrap, 5 ml column.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 ml	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

Medium properties

Glutathione Sepharose 4B is designed for purification of glutathione S-transferase (GST) tagged proteins produced using the pGEX series of expression vectors, other glutathione S-transferases, and glutathione binding proteins. GST-tagged proteins can be purified directly from pretreated bacterial lysates with a one-step method using GSTrap 4B. The tagged proteins are eluted under mild, non-denaturing conditions that preserve protein antigenicity and function. The glutathione ligand is coupled via a 10-carbon linker to 4% agarose. The coupling is optimized to give high binding capacity for GST-tagged proteins and other glutathione binding proteins.

The total binding capacity for GST-tagged proteins depends on size, conformation, and concentration of the protein in the sample. The dynamic binding capacity will also vary depending on the flow rate. The GST tag (a naturally occurring M_r 26 000 protein) can be removed by treatment with the appropriate site-specific protease. Proteolytic cleavage can be performed while the tagged protein is bound to GSTrap 4B or, alternatively, after elution. Cleavage on GSTrap 4B eliminates the extra step of separating the released protein from GST, since the GST-tag remains bound. The target protein is eluted using binding buffer.

The characteristics of GSTrap 4B are summarized in Table 2.

Table 2. GSTrap 4B characteristics

Ligand	glutathione and 10-carbon linker arm
Ligand concentration	7 to 15 µmol glutathione/ml medium
Binding capacity[1]	Approx. 25 mg recombinant glutathione S-transferase (M_r 26 000)/ml medium (protein dependent)
Mean particle size	90 µm
Bead structure	4% agarose
Recommended flow rates[1]	Sample loading: 0.2 to 1 ml/min (1 ml) and 0.5 to 2 ml/min (5 ml); Washing and elution: 1 ml/min (1 ml) and 5 ml/min[2] (5 ml)
Chemical stability	All commonly used aqueous buffers, e.g., 1 M acetate pH 4.0 and 6 M guanidine hydrochloride for 1 h at room temperature
pH stability	4 to 13
Storage temperature	4°C to 30°C
Storage	20% ethanol

[1] 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] **Note:** Recommended flow rate during washing and elution for GSTrap 4B 5 ml column when running at 4 to 8°C is up to 4 ml/min.

2 Operation

The columns can be operated with a syringe, peristaltic pump, or a liquid chromatography system.

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. We recommend filtering the buffers by passing them through a 0.45 µm filter before use.

Binding buffer

PBS, pH 7.4 (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4)

Elution Buffer

50 mM Tris-HCl, 10 to 20 mM reduced glutathione, pH 8.0

Note: *1 to 20 mM DTT may be included in the binding and elution buffers to increase the purity. However, this may result in lower yield of GST-tagged protein.*

Sample preparation

The sample should be centrifuged and/or filtered through a 0.45 µm filter immediately before it is applied to the column. If the sample is too viscous, dilute it with binding buffer to prevent clogging the column. Less protein may bind to the medium due to a lower protein concentration in the sample.

Purification

1. Fill the pump tubing or syringe with binding buffer. Connect the column to the syringe (use the connector supplied) or pump tubing "drop to drop" to avoid introducing air into the column.
2. Remove the snap-off end at the column outlet.
3. Equilibrate the column with 5 column volumes of binding buffer using up to 1 ml/min (1 ml column) and up to 2.5 ml/min (5 ml column).

4 Apply the sample using a syringe fitted to the Luer connector or by pumping it onto the column. For best results, use a flow rate of 0.2 to 1 ml/min (1 ml column) and 0.5 to 2 ml/min (5 ml column) during sample application.

5 Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent. A flow rate of 1 ml/min (1 ml column) and 5 ml/min (5 ml column) is recommended for washing.

Note: *If GSTrap 4B 5 ml column is run at 4°C to 8°C the recommended flow rate during washing is up to 4 ml/min.*

6 Elute with 5 to 10 column volumes of elution buffer. A flow rate of 1 ml/min (1 ml column) and 5 ml/min (5 ml column) is recommended for elution.

Note: *If GSTrap 4B 5 ml column is run at 4°C to 8°C the recommended flow rate during elution is up to 4 ml/min.*

Notes

- One parameter affecting the binding of GST-tagged proteins or other glutathione binding proteins to GSTrap 4B is flow rate. Due to the relatively slow binding kinetics between GST and glutathione, it is important to keep the flow rate low during sample application for maximum binding capacity. Protein characteristics, pH, and temperature are other factors that may affect the binding capacity. However, when working with sensitive proteins, higher flow rates are recommended to minimize purification time.
- Volumes and times used for elution may vary among tagged proteins. Additional elutions with higher concentrations of glutathione may be required. Flowthrough, wash, and eluted material from the column should be monitored for GST-tagged proteins using SDS-PAGE in combination with Western blotting, if necessary.
- The GST Detection Module can be used to optimize conditions for elution or to trace steps in the purification of a GST-tagged protein. The Module is designed to identify GST-tagged proteins using either a biochemical or an immunological assay.

- The concentration of GST-tagged protein can be estimated by measuring the absorbance at 280 nm. The GST-tag can be approximated using the conversion; A_{280} ≈1 corresponds to 0.5 mg/ml.
- The concentration of GST-tagged protein may also be determined by standard chromogenic methods (e.g. Lowry, BCA™, and Bradford assays). If Lowry or BCA assays are to be used, the sample must first be buffer exchanged using a HiTrap Desalting 5 ml column, a HiPrep™ 26/10 Desalting column, or dialyzed against PBS to remove glutathione, which can interfere with the protein measurement. The Bradford method can be used in the presence of glutathione.
- The reuse of GSTrap 4B depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.

Cleaning GSTrap 4B

If the chromatography medium appears to be losing binding capacity, it may be due to an accumulation of precipitate, denatured, or non-specifically bound proteins.

Removal of precipitated or denatured substances

- Wash with 2 column volumes of 6 M guanidine hydrochloride, immediately followed by 5 column volumes of PBS.

Removal of hydrophobically bound substances

- Wash with 3 to 4 column volumes of 70% ethanol or 2 column volumes of 1% Triton™ X-100 immediately followed by 5 column volumes of PBS.

3 Scaling up

Scaling up is easily done going from a GSTrap 4B 1 ml column to a 5 ml column. Glutathione Sepharose 4B is available in lab packs for packing larger columns.

4 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 3. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

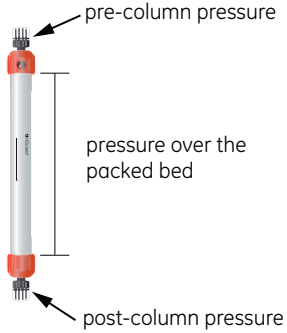

Fig 3. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

1. Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2. Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3. Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

   The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

5 Storage

Store the column at 4°C to 30°C in 20% ethanol.

6 Cleavage of GST-tagged proteins

If removal of the GST-tag is necessary, tagged proteins containing a PreScission™ Protease recognition site, a thrombin recognition site, or a factor Xa recognition site may be cleaved either while bound to GSTrap 4B or in solution after elution. Cleavage after elution is suggested if optimization of cleavage conditions is necessary. Samples can easily be removed at various time points and analyzed by SDS-PAGE to estimate the yield, purity, and extent of digestion. The amount of protease used, the temperature, and the length of incubation required for complete digestion may vary depending on the tagged protein. Optimal conditions for each tagged-protein should be determined in pilot experiments, for example, incubation time may be reduced by using higher concentrations of proteolytic enzyme.

PreScission Protease

PreScission Protease, M_r 46 000

PreScission cleavage buffer

50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol (DTT), pH 7.5

PreScission Protease cleavage of GST-tagged protein bound to GSTrap 4B

Assumption: 8 mg GST-tagged protein bound/ml medium

1. Follow steps 1 to 5 in Section 2. Operation: "Purification", on page 6.
2. Wash GSTrap 4B with 10 column volumes of PreScission cleavage buffer.
3. Prepare the PreScission Protease mix: GSTrap 4B 1 ml column (8 mg GST-tagged protein bound): Mix 80 µl (160 units) of PreScission Protease with 920 µl of PreScission cleavage buffer at 4°C. GSTrap 4B 5 ml column (40 mg GST-tagged protein bound): Mix 400 µl (800 units) of PreScission Protease with 4.6 ml of PreScission cleavage buffer at 4°C.

4 Load the PreScission Protease mix onto the column using a syringe and the connector supplied. Seal the column with the top and bottom stop plugs supplied.

5 Incubate the column at 4°C for 4 h.

6 Fill a syringe with 3 ml (1 ml column) or 15 ml (5 ml column) of PreScission cleavage buffer. Remove the top and bottom stop plugs. Avoid introducing air into the column. Elute the column and collect the eluate (0.5 ml-1 ml/tube). The eluate will contain the protein of interest, while the GST moiety of the tagged protein and the PreScission Protease will remain bound to GSTrap 4B.

PreScission Protease cleavage of eluted GST-tagged protein

Assumption: 8 mg GST-tagged protein bound/ml medium

1 Follow steps 1 to 5 in Section 2. Operation: "Purification", on page 6.

2 Remove the reduced glutathione from the eluate using a quick buffer exchange on a HiTrap Desalting, PD-10 Desalting, or HiPrep 26/10 Desalting column, depending on the sample volume, or dialyze against PreScission cleavage buffer.

3 Add 1 µl (2 U) of PreScission Protease for each 100 µg of tagged protein in the eluate. If the amount of tagged protein in the eluate has not been determined, add 80 µl (160 units) of PreScission Protease for tagged protein eluted from GSTrap 4B 1 ml column; add 400 µl (800 units) of PreScission Protease for tagged protein eluted from GSTrap 4B 5 ml column.

4 Incubate at 4°C for 4 h.

5 Once digestion is complete, apply the sample to an equilibrated GSTrap 4B column to remove the GST moiety of the tagged protein and the PreScission Protease. The protein of interest will be found in the flowthrough, while the GST moiety of the tagged protein and the PreScission Protease will remain bound to GSTrap 4B.

Thrombin

Thrombin, M_r 37 000

Thrombin cleavage buffer

PBS, pH 7.4 (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3)

Preparation of thrombin solution

1 Dissolve 500 U thrombin in cold 500 µl PBS (1 U/µl).

2 Swirl gently to dissolve.

3 Freeze as 80 µl aliquots and keep at –80°C.

Thrombin cleavage of GST-tagged protein bound to GSTrap 4B

Assumption: 8 mg GST-tagged protein bound/ml medium

1 Follow steps 1 to 5 in Section 2. Operation: "Purification", on page 6.

2 Prepare the thrombin mix: GSTrap 4B 1 ml column (8 mg GST-tagged protein bound): Mix 80 µl thrombin solution (1 U/µl) with 920 µl PBS. GSTrap 4B 5 ml column (40 mg GST-tagged protein bound): Mix 400 µl thrombin solution with 4.6 ml PBS.

3 Load the thrombin solution onto the column using a syringe and the connector supplied. Seal the column with the top and bottom plugs supplied.

4 Incubate the column at room temperature (22°C to 25°C) for 2 to 16 h.

5 Fill a syringe with 3 ml (1 ml column) or 15 ml (5 ml column) PBS. Remove the top and bottom stop plugs from the column. Avoid introducing air into the column. Elute the column and collect the eluate (0.5 ml-1 ml/ tube). The eluate will contain the protein of interest and thrombin, while the GST moiety of the tagged protein will remain bound to GSTrap 4B.

Note: *After cleavage using thrombin, the enzyme can be removed from eluted protein using HiTrap Benzamidine FF (high sub), see "Ordering information".*

Thrombin cleavage of eluted GST-tagged protein

Assumption: 8 mg GST-tagged protein bound/ml medium

1. Follow steps 1 to 5 in Section 2. Operation: "Purification", on page 6.
2. Add 10 µl (10 units) of thrombin solution for each mg of tagged protein in the eluate. If the amount of tagged protein in the eluate has not been determined, add 80 µl (80 U) thrombin solution for tagged protein eluted from GSTrap 4B 1 ml column; add 400 µl (400 U) thrombin solution for tagged protein eluted from GSTrap 4B 5 ml column.
3. Incubate at room temperature (22°C to 25°C) for 2 to 16 h.
4. Once digestion is complete, GST can be removed by first removing glutathione using a quick buffer exchange on a HiTrap Desalting, PD-10 Desalting, or HiPrep 26/10 Desalting column depending on the sample volume, or dialyzing against PBS. Then apply the sample to an equilibrated GSTrap 4B column. The purified protein of interest and thrombin will be found in the flowthrough.

Note: *After cleavage using thrombin, the enzyme can be removed from eluted protein using HiTrap Benzamidine FF (high sub), see "Ordering information".*

Factor Xa

Factor Xa, M_r 48 000

Note: *Factor Xa consists of two subunits linked by disulfide bridges. As glutathione can disrupt disulfide bridges, it should be removed from the sample prior to the cleavage reaction. Factor Xa cleavage buffer: 50 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.5*

Preparation of factor Xa solution

1. Dissolve 400 U factor Xa in 400 µl cold water (1 U/µl).
2. Swirl gently to dissolve.
3. Freeze as 80 µl aliquots and keep at –80°C.

Factor Xa cleavage of GST-tagged protein bound to GSTrap 4B

Assumption: 8 mg GST-tagged protein bound/ml medium

1. Follow steps 1 to 5 in Section 2. Operation: "Purification", on page 6.
2. Wash GSTrap 4B with 10 column volumes of factor Xa cleavage buffer.
3. Prepare the factor Xa mix: GSTrap 4B 1 ml column (8 mg GST-tagged protein bound): Mix 80 µl factor Xa solution with 920 µl factor Xa cleavage buffer. GSTrap 4B 5 ml column (40 mg GST-tagged protein bound): Mix 400 µl factor Xa solution with 4.6 ml factor Xa cleavage buffer.
4. Load the mix onto the column using a syringe and the connector supplied. Seal the column with the top and bottom stop plugs supplied.
5. Incubate the column at room temperature (22°C to 25°C) for 2 to 16 h.

6 Fill a syringe with 3 ml (1 ml column) or 15 ml (5 ml column) factor Xa cleavage buffer. Remove the top and bottom stop plugs from the column. Avoid introducing air into the column. Elute the column and collect the eluate (0.5 ml-1 ml/tube). The eluate will contain the protein of interest and factor Xa, while the GST moiety of the tagged protein will remain bound to GSTrap 4B.

Note: *After cleavage using factor Xa the enzyme can be removed from eluted protein using HiTrap Benzamidine FF (high sub), see "Ordering information".*

Factor Xa cleavage of eluted GST-tagged protein

Assumption: 8 mg GST-tagged protein bound/ml medium

1 Follow steps 1 to 5 in Section 2. Operation: "Purification", on page 6.

2 Remove the reduced glutathione from the eluate using a quick buffer exchange on a HiTrap Desalting, PD-10 Desalting, or HiPrep 26/10 Desalting column depending on sample volume, or dialyze against factor Xa cleavage buffer.

3 Add 10 units of factor Xa solution for each milligram tagged protein in the eluate. If the amount of tagged protein in the eluate has not been determined, add 80 µl (80 units) of factor Xa solution for eluted tagged protein from GSTrap 4B 1 ml column; add 400 µl (400 units) of factor Xa solution for eluted tagged protein from GSTrap 4B 5 ml column.

4 Incubate the column at room temperature (22°C to 25°C) for 2 to 16 h.

5 Once digestion is complete, apply the sample to an equilibrated GSTrap 4B column to remove the GST moiety. The protein of interest will be found in the flowthrough together with factor Xa.

Note: *After cleavage using factor Xa the enzyme can be removed from eluted protein using HiTrap Benzamidine FF (high sub), see ordering information.*

7 Troubleshooting guide

Consult the GST Gene Fusion System Handbook for more detailed information, see "Ordering information" and pGEX instructions regarding troubleshooting recommendations for expression, fermentation, and solubilization.

Symptom	Possible cause	Remedy
GST-tagged protein does not bind to GSTrap 4B	Column not equilibrated	Equilibrate GSTrap 4B before use. Check that the GSTrap 4B column has been equilibrated with a buffer pH 6.5 to 8.0 (e.g. PBS) before the cell lysate is applied. Binding of GST-tagged proteins to GSTrap 4B is not efficient at pH less than 6.5 or greater than 8.
	Flow rate too high	Decrease flow rate during sample load, see note under Section 2. Operation: "Purification" on page 6.
	Too extensive sonication/ mechanical lysis denatured the tagged protein, which is preventing binding	Use mild conditions during cell lysis.
	Reducing agent missing	Add DTT to a final concentration of 1 to 20 mM prior to cell lysis. This may increase binding of some GST-tagged proteins to GSTrap 4B.
	Tagged protein concentration is too dilute	Concentrate the sample. The binding capacity is concentration dependent. Low expressed proteins may not bind as efficiently as high expressed proteins. Therefore, concentrate the sample to improve binding.

Symptom	Possible cause	Remedy
	Target protein may have altered conformation of GST, thereby reducing affinity for the medium	Test the binding of GST from the parental expression vector. Prepare a lysate of cells thereby reducing affinity harboring the parental GST vector and check binding to the medium. If GST produced from the parental vector binds with high affinity, 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 column washing.
	GSTrap 4B column needs cleaning	See Section 2. Operation: "Cleaning GSTrap 4B". If the GSTrap 4B column has already been used several times, it may be necessary to use a new GSTrap 4B column.
GST-tagged protein is not eluted efficiently from GSTrap 4B	Glutathione concentration is too low	Increase the concentration of glutathione in the elution buffer. The 10 to 20 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.
	Insufficient time for elution	Increase the time used for elution. Decrease the flow during elution.
	Insufficient volume of elution buffer	Increase the volume of elution buffer. Sometimes, especially after on-column cleavage of tagged protein, a larger volume of buffer may be necessary to elute the target protein.
	pH is too low	Increase the pH of the elution buffer. A low pH may limit elution from GSTrap 4B. Increasing the pH of the elution buffer to pH 8 to 9 may improve elution without requiring an increase in the concentration of glutathione used for elution.
	Ionic strength is too low	Increase the ionic strength of the elution buffer. Adding 0.1 to 0.2 M NaCl to the elution buffer may also improve results.

Symptom	Possible cause	Remedy
	Non-specific hydrophobic interactions are interfering with elution	Add a non-ionic detergent to the elution buffer. Non-specific hydrophobic interactions may prevent solubilization and elution of tagged proteins from GSTrap 4B. Adding 0.1% Triton X-100 or 2% N-octylglucoside can significantly improve elution of some GST-tagged proteins.
Multiple bands are observed after electro-phoresis/ Western blotting analysis of eluted target protein	M_r 70 000 protein is co-purifying 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 GSTrap 4B. Alternatively, remove the DnaK protein by passing the tagged protein solution through ATP-agarose or by ion exchange.
	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.
	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 provided with the pGEX vectors. This strain is *ompT*.

Symptom	Possible cause	Remedy
	Over-sonication can cause co-purification of host proteins with GST-tagged proteins	Decrease sonication or change to another lysis method (e.g., French press). Cell disruption is apparent by partial clearing of the suspension protein 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 sonication may improve results. Avoid frothing as this may denature the tagged protein.
	Chaperonins are co-purifying with GST-tagged protein	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.
	Antibody used for detection in Western blotting may be cross-reacting with *E. coli* host proteins present in 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* lysate 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.

Symptom	Possible cause	Remedy
Incomplete cleavage of GST-tagged proteins	The PreScission Protease, thrombin, or factor Xa to tagged protein ratios are incorrect	Check the amount of tagged protein in the digestion. Note that the capacity of GSTrap 4B for GST is approximately 10 mg/ml chromatography medium. In most purifications, however, the matrix is not saturated with tagged protein. Use the following ratios: • PreScission protease: at least 10 units/mg tagged protein. • Thrombin: at least 10 units/mg tagged protein. One cleavage unit of thrombin from GE Healthcare digests 90% of 100 µg of a test tagged protein in 16 h at 22°C. • Factor Xa: at least 1% (w/w) tagged protein. For some tagged proteins, up to 5% factor Xa can be used. The optimum amount must be determined empirically. In some cases, a tagged protein concentration of 1 mg/ml has been found to give optimal results.
	Insufficient incubation time	Increase incubation time and enzyme concentration. For PreScission Protease, thrombin or factor Xa, increase the reaction time to 20 h or more if the tagged protein is not degraded by extensive incubation. The amount of enzymes can also be increased.
	Cleavage site sequence altered	Verify the presence of specific cleavage sites. Check the DNA sequence of the vector construct. Compare it with a known sequence and verify that the specific cleavage sites for the enzyme used have not been altered during the cloning of your tagged protein.
	Sample contains protease inhibitors	Make sure that protease inhibitors are absent by performing a buffer exchange of the tagged protein against the cleavage buffer. See Section 6. Cleavage of GST-tagged proteins, for cleavage buffer compositions.

Symptom	Possible cause	Remedy
	Factor Xa cleavage reaction may need SDS	Adding 0.5% (w/v) SDS to the reaction buffer can significantly improve factor Xa cleavage with some tagged proteins. Various concentrations of SDS should be tested to find the optimum concentration.
	Factor Xa is not properly activated	Activate factor Xa. Functional factor Xa requires activation of factor X with Russell's viper venom. Activation conditions are a ratio of Russell's viper venom to factor Xa of 1% in 8 mM Tris-HCl, 70 mM NaCl, 8 mM $CaCl_2$, pH 8.0. Incubate at 37°C for 5 min. Factor Xa from GE Healthcare has been preactivated by this procedure.
	The first amino acid after the factor Xa recognition sequence is Arg or Pro	Check the sequence of the fusion partner to be sure that the first three nucleotides after the factor Xa recognition sequence do not code for Arg or Pro.
Multiple bands are observed on SDS gels following enzyme cleavage	Proteolysis occurred in 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 sequence of the cloned protein for protease recognition sequences. See the *GST Gene Fusion System Handbook* for details.

8 Ordering information

Product	Quantity	Code No
GSTrap 4B	1 x 1 ml	29-0486-09
	5 x 1 ml	28-4017-45
	100 x 1 ml[1]	28-4017-46
	1 x 5 ml	28-4017-47
	5 x 5 ml	28-4017-48
	100 x 5 ml[1]	28-4017-49

[1] Pack size available by specific customer order.

Related product	Quantity	Code No.
Glutathione Sepharose 4B	10 ml	17-0756-01
	100 ml	17-0756-05
	300 ml	17-0756-04
HiTrap Benzamidine FF (high sub)	5 × 1 ml	17-5143-01
	2 × 1 ml	17-5143-02
	1 × 5 ml	17-5144-01
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
	100 x 5 ml[1]	11-0003-29
PD-10 Desalting columns	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
GST Detection Module	50 reactions	27-4590-01
Anti-GST Antibody	0.5 ml	27-4577-01
E. coli BL21	1 vial	27-1542-01

[1] Pack size available by specific customer order.

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

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column)*	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related 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
Affinity Chromatography Columns and Media, Selection guide	18-1121-86
Prepacked Chromatography Columns for ÄKTAdesign Systems, Selection guide	28-9317-78

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