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GSTrap HP, 1 ml and 5 ml

Instructions for Use

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71502755 AG

Abstract

GSTrap™ HP 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 pre-treated bacterial lysates using GSTrap HP. Tagged proteins are eluted under mild, non-denaturing conditions that preserve protein antigenicity and function. The medium, Glutathione Sepharose™ High Performance, is also available as lab packages and is an excellent choice for high performance purifications.
The columns can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™.

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Important

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 x 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 High Performance is designed for the purification of glutathione S-transferase (GST) tagged proteins produced using the pGEX series of expression vectors (1), other glutathione S-transferases and glutathione-binding proteins. GST-tagged proteins can be purified directly from bacterial lysates with a one-step method using GSTrap HP. 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 highly crosslinked 6% agarose. The coupling is optimized to give high binding capacity for GST-tagged proteins and other glutathione-binding proteins.

The total binding capacity is approx. 10 mg pure GST-tagged protein/ml medium. The dynamic binding capacity will vary depending of several factors, such as target protein, flowrate, etc. If removal of the GST-tag (a naturally occurring M_r 26 000 protein) is required, the tagged protein can be digested with the appropriate site-specific protease while bound to GSTrap HP or, alternatively, after elution. Cleavage on GSTrap HP 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.

Table 2. GSTrap HP characteristics

Ligand	Glutathione and 10-carbon linker arm
Ligand concentration	1.5–3.5 mg glutathione/ml medium (based on Gly)
Dynamic binding capacity[2]	Approx. 7 mg GST-tagged protein/ml medium, M_r 63 000
Mean particle size	34 µm
Bead structure	Highly cross-linked 6% agarose
Maximum flow rate	4 ml/min and 15 ml/min for 1 and 5 ml columns respectively
Recommended flow rates[1]	Sample loading: 0.2 to 1 ml/min (1 ml) and 1 to 5 ml (5 ml) Washing and elution: 1 to 2 ml/min (1 ml) and 5 to 10 ml/min (5 ml)
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	pH 3 to 12
Storage temperature	4°C to 30°C
Storage	20% ethanol

[1] Note: Binding of GST to glutathione is flow rate dependent and lower flow rates often increase the binding capacity. This is important during sample loading and elution.

[2] Dynamic binding capacity conditions (60% breakthrough)

Sample:	1 mg/ml pure GST-tagged protein in binding buffer
Column	0.4 ml
Flow rate:	0.2 ml/min (60 cm/h)
Binding buffer:	10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

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

Elution Buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

Note: *1 to 10 mM DTT can be included in the binding and elution buffer,* see *p. 13.*

Sample preparation

The sample should be centrifuged and/or filtered through a 0.22 µm or 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.

Purification

1. Fill the pump tubing or syringe with binding buffer. Connect the column to the syringe (use the supplied luer connector) 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.
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 1 to 5 ml/min (5 ml column) during sample application.
5. Wash with approx. 5 column volumes of binding buffer or until no material appears in the effluent. A flow rate of 1 to 2 ml/min

(1 ml column) and 5 to 10 ml/min (5 ml column) is recommended for washing.

6 Elute with 3–5 column volumes of elution buffer. A flow rate of 1 to 2 ml/min (1 ml column) and 5 to 10 ml/min (5 ml column) is recommended for elution.

Note:

- One of the most important parameters affecting the binding of GST-tagged proteins or other glutathione binding proteins to GSTrap HP is the 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.
- Volumes and times used for elution may vary among tagged proteins. Additional elutions with higher concentrations of glutathione may be required. Flow through, wash and eluted material from the column should be monitored for GST-tagged proteins using SDS-PAGE in combination with Western Blot if necessary.
- The GST Detection Module (see "Ordering information") 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 following conversion: A280 ^{a} 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, 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 HP depends on the nature of the sample and should only be performed with identical samples to

prevent cross-contamination.

Cleaning GSTrap HP

If the 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

For quick scale-up of purifications, two or three GSTrap HP can be connected in series (backpressure will increase). Further scaling up is easy using the lab packages.

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.*

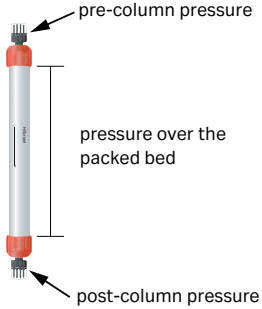

Fig 3. Pre-column and post-column measurements.

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, fusion 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 HP 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 fusion protein. Optimal conditions for each fusion protein should be determined in pilot experiments, e.g. incubation time may be reduced by using higher concentrations of proteolytic enzyme.

1 PreScission Protease

PreScission Protease, M_r 46 000

(The PreScission Protease includes a GST-tag)

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 HP

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

1 Follow steps 1 to 5 under "Purification", (see p. 7).

2 Wash GSTrap HP with 10 column volumes of PreScission cleavage buffer.

3 Prepare the PreScission Protease mix:

- GSTrap HP 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 HP 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 adaptor supplied. Seal the column with the top cap and the stop plug supplied.

5 Incubate the column at 4°C for 4 hours.

6 Fill a syringe with 3 ml (1 ml column) or 15 ml (5 ml column) of PreScission cleavage buffer. Remove the top cap and domed nut. Avoid introducing air into the column. Elute the column and collect the eluate (0.5 ml to 1 ml/tube). The eluate will contain the protein of interest, while the GST moiety of the fusion protein and the PreScission Protease will remain bound to GSTrap HP.

PreScission Protease cleavage of eluted GST-tagged protein

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

1 Follow steps 1 to 6 under "Purification", (see p. 7).

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

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 (tagged protein eluted from GSTrap HP 1 ml column) or 400 µl (800 units) of PreScission Protease (tagged protein eluted from GSTrap HP 5 ml column).

4 Incubate at 4°C for 4 hours.

5 Once digestion is complete, apply the sample to an equilibrated GSTrap HP 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 HP

2 Thrombin

Thrombin, M_r 37 000

Thrombin cleavage buffer: PBS, 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 HP

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

1. Follow steps 1 to 5 under "Purification", (see p. 7).
2. Prepare the thrombin mix:
   - GSTrap HP 1 ml column (8 mg GST-tagged protein bound): Mix 80 µl thrombin solution (1 U/µl) with 920 µl PBS.
   - GSTrap HP 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 adaptor supplied.

   Seal the column with the top cap and the domed nut supplied.
4. Incubate the column at room temperature (22°C to 25°C) for 2 to 16 hours.
5. Fill a syringe with 3 ml (1 ml column) or 15 ml (5 ml column) PBS. Remove the top cap and stop plug from the column. Avoid introducing air into the column. Elute the column and collect the eluate (0.5 ml to 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 HP.

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 6 under "Purification", (see p. 7).

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 (tagged protein eluted from GSTrap HP 1 ml column) or 400 µl (400 U) thrombin solution (tagged protein eluted from GSTrap HP 5 ml column).

3 Incubate at room temperature (22°C to 25°C) for 2 to 16 hours.

4 Once digestion is complete, remove glutathione by using a quick buffer exchange on HiTrap Desalting, a PD-10 column or HiPrep 26/10 Desalting depending on the sample volume, or dialyzing against PBS to remove GST bound glutathione.

Then apply the sample to an equilibrated GSTrap HP column for the removal of remaining GST. The purified protein of interest and thrombin will be found in the flow-through.

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

3 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 HP

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

1 Follow steps 1 to 5 under "Purification", (see p. 7).

2 Wash GSTrap HP with 10 column volumes of factor Xa cleavage buffer.

3 Prepare the factor Xa mix:
GSTrap HP 1 ml column (8 mg GST-tagged protein bound):
Mix 80 µl factor Xa solution with 920 µl factor Xa cleavage buffer.
GSTrap HP 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 cap and the stop plug supplied.

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

6 Fill a syringe with 3 ml (1 ml column) or 15 ml (5 ml column) factor Xa cleavage buffer. Remove the top cap and stop plug from the column. Avoid introducing air into the column. Elute the column and collect the eluate (0.5 ml to 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 HP.

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 6 under "Purification", (see p. 7).
2. Remove the reduced glutathione from the eluate using a quick buffer exchange on HiTrap Desalting, a PD-10 Desalting column or HiPrep 26/10 Desalting depending on sample volume, or dialyze against factor Xa cleavage buffer to remove GST bound glutathione.
3. Add 10 units of factor Xa solution for each mg 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 (eluted tagged protein from GSTrap HP 1 ml column) or 400 µl (400 units) of factor Xa solution (eluted tagged protein from GSTrap HP 5 ml column).
4. Incubate the column at room temperature (22°C to 25°C) for 2 to 16 hours.
5. Once digestion is complete, apply the sample to an equilibrated GSTrap HP column to remove the GST moiety of the fusion. The protein of interest will be found in the flow-through 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

Consult the *GST Gene Fusion System Handbook* (ref. 1) for more detailed information and pGEX instructions regarding troubleshooting recommendations for expression, fermentation and solubilization.

GST-tagged protein does not bind to GSTrap HP

- **GST-tagged protein denatured by sonication:** Too extensive sonication can denature the tagged protein and prevent it binding to GSTrap HP. Use mild sonication conditions during cell lysis. Conditions for sonication must be empirically determined.
- **Add DTT prior to cell lysis and to buffers:** Adding DTT to a final concentration of 1 to 10 mM may significantly increase binding of some GST tagged proteins to GSTrap HP.
- **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 its affinity. Adequate results may be obtained by reducing the temperature used for binding to 4°C, and by limiting column washing.
- **Equilibrate GSTrap HP before use:** Binding of GST tagged proteins to GSTrap HP is not efficient at pH less than 6.5 or greater than 8. Check that the GSTrap HP column has been equilibrated with a buffer pH 6.5 to 8.0 (e.g., PBS) before the clarified cell lysate is applied.
- **Use a fresh GSTrap HP:** If the GSTrap HP column has already been used several times, it may be necessary to use a new GSTrap HP column. See also "Cleaning GSTrap HP".
- **Decrease flow rate during sample load.** See Note on p. 8.

GST-tagged protein is not eluted efficiently from GSTrap HP

- **Increase the time used for elution:** Decrease the flow during elution.
- **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 tagged protein.
- **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.
- **Increase the pH of the elution buffer:** A low pH may limit elution from GSTrap HP. 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.
- **Increase the ionic strength of the elution buffer:** Adding 0.1 to 0.2 M NaCl to the elution buffer may also improve results.
- **Use fresh elution buffer (reduced glutathione)**.
- **Add a non-ionic detergent to the elution buffer:** Non-specific hydrophobic interactions may prevent solubilization and elution of tagged proteins from GSTrap HP. 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 GSTrap HP.

  Alternatively, remove the DnaK protein by passing the tagged protein solution through ATP-agarose or by ion exchange.

- **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 Cytiva*

- **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.
- **Decrease sonication:** 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 sonication may improve results. Avoid frothing as this may denature the fusion protein. Over-sonication can also lead to the co-purification of host proteins with the 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.
- **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 Cytiva has been cross-adsorbed against *E. coli* proteins and tested for its lack of non-specific background binding in Western Blots.

Incomplete cleavage of GST-tagged proteins

- **The PreScission Protease, thrombin or factor Xa to fusion protein ratios are incorrect:** Check the amount of tagged protein in the digestion. Note that the capacity of GSTrap HP for GST is approx. 10 mg/ml medium. In most purifications, however, the matrix is not saturated with tagged protein.

Ratios: **PreScission protease,** at least 10 units/mg tagged protein. Thrombin, at least 10 units/mg tagged protein. One cleavage unit of thrombin from Cytiva digests. ≥ 90% of 100 µg of a test tagged protein in 16 hours 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. Adding. 0.5% (w/v) 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.

- **Increase incubation time and enzyme concentration:** For PreScission Protease, thrombin or factor Xa, increase the reaction time to 20 hours or more if the tagged protein is not degraded by extensive incubation. The amount of enzymes can also be increased.
- **Verify the presence of specific cleavage sites:** Check the DNA sequence of the construct. Compare it with a known sequence and verify that the different specific cleavage sites for the enzyme used have not been altered during the cloning of your tagged protein.

Make sure that cleavage enzyme inhibitors are absent:

- **PreScission Protease:** Buffer exchange or dialyze the tagged protein against 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.5 before cleavage.

- **Factor Xa:** Buffer exchange on HiTrap Desalting, a PD-10 column or HiPrep 26/10 Desalting depending on the sample volume, or dialyze against 50 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.5.
- **Factor Xa is not properly activated:** 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 Cytiva 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 tagged 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 after electrophoresis analysis of cleaved target protein:

- **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 *GST Gene Fusion System Handbook* (ref. 1) for details.

8 Reference

1. GST Gene Fusion System Handbook, GE Healthcare, Product code 18-1157-58

For more details about HiTrap columns visit *cytiva.com/hitrap*.

9 Ordering information

Product	Quantity	Product code
GSTrap HP	5 × 1 ml	17-5281-01
	100 × 1 ml[1]	17-5281-05
	1 × 5 ml	17-5282-01
	5 × 5 ml	17-5282-02
	100 × 5 ml[1]	17-5282-05

Related products	Quantity	Product code
Glutathione Sepharose High Performance	25 ml	17-5279-01
	100 ml[2]	17-5279-02
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
	100 × 5 ml[1]	11-0003-29
HiTrap Benzamidine FF (high sub)	5 × 1 ml	17-5143-01
	2 × 1 ml	17-5143-02
	1 × 5 ml	17-5144-01
PD-10 Desalting column	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

[1] Special pack delivered on specific customer order.

[2] Larger quantities are available. Please contact Cytiva for more information.

Site-Specific Proteases

Product	Quantity	Product code
PreScission Protease	500 units	27-0843-01
Thrombin	500 units	27-0846-01
Factor Xa	400 units	27-0849-01

Companion Products

Product	Quantity	Product code
GST Detection Module	50 reactions	27-4590-01
Anti-GST Antibody	0.5 ml	27-4577-01

Accessories	Quantity	Product code
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

Literature	Product code
GST Gene Fusion System Handbook	18-1157-58
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
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