Third Edition **December 2014**

Glutathione Affinity Handbook

For purifying and detecting proteins carrying a GST tag

Glutathione HiCap Matrix

Glutathione HiCap Cartridges

GST-tag Antibody

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Contents

Kit Contents

Glutathione HiCap Matrix	**(10 ml)**	**(100 ml)**	**(500 ml)**
Catalog no.	**30900**	**30930**	**30950**
Glutathione HiCap Matrix resin (bed volume)	10 ml	100 ml	5 x 100 ml
Handbook	1	1	1

Glutathione HiCap Cartridge	**(5 x 1 ml)**	**(5 x 5 ml)**
Catalog no.	**30971**	**30991**
Number of preps	***5***	***5***
Glutathione HiCap Cartridges	5	5
Handbook	1	1

GST-tag Antibody	**(100 µg)**
Catalog no.	**34860**
GST-tag Antibody (lyophilized)	100 µg
Handbook	1

Shipping and Storage

Glutathione HiCap Matrix and Glutathione HiCap Cartridges are shipped at ambient temperature and should be stored at 2–8°C. Under these conditions, Glutathione HiCap matrices can be stored for at least 12 months without any reduction in performance.

GST-tag Antibodies are shipped at ambient temperature. GST-tag Antibodies should be stored lyophilized until use. They can be stored lyophilized for at least 12 months at 2–8°C to 20°C. In solution, they can be stored for 3 months at 2–8°C or for at least 12 months in aliquots at –80°C. Avoid repeated freezing and thawing.

Product Use Limitations

The kits and reagents described in this handbook are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding Glutathione HiCap Matrix, Glutathione HiCap Cartridges, GST-tag Antibodies, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Glutathione HiCap Matrix, Glutathione HiCap Cartridges, and GST-tag Antibody is tested against predetermined specifications to ensure consistent product quality.

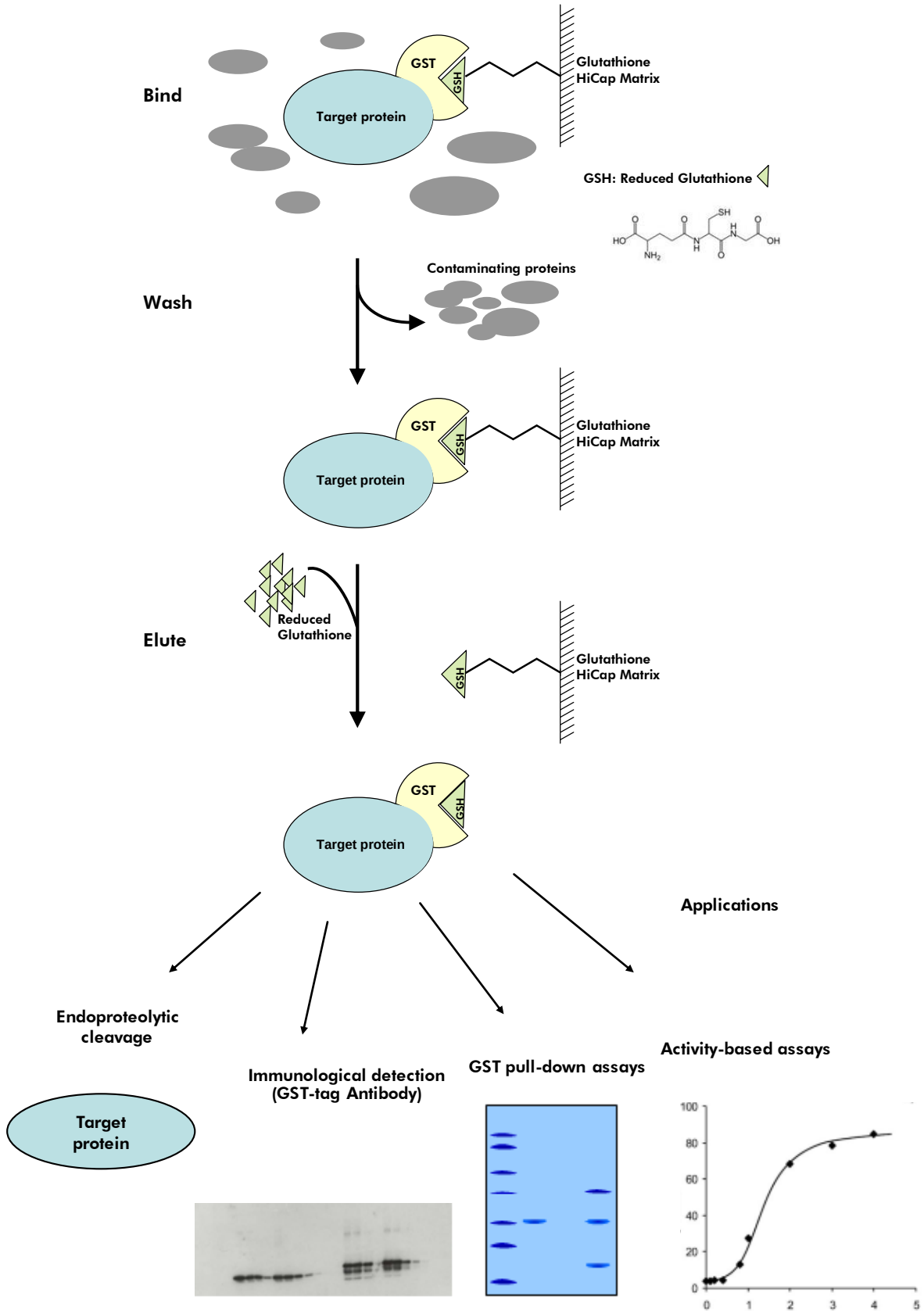
Introduction

Glutathione affinity is an efficient method for single-step purification of proteins carrying a GST (glutathione S-transferase) tag fusion. GST protein from various sources, both native and recombinantly expressed in *E. coli* and other host cells, can be purified by affinity chromatography on immobilized glutathione followed by competitive elution with excess reduced glutathione (1–3). Smith and Johnson (4) made use of this observation when they recombinantly fused the coding regions of GST from *Schistosoma japonicum* and a protein of interest for subsequent glutathione affinity purification of heterologously expressed GST-tagged proteins.

GST can be solubly expressed in *E. coli* cytoplasm in high amounts and with full enzymatic activity. Furthermore, many eukaryotic proteins that are insoluble when expressed in *E. coli* have shown to be at least partially soluble when expressed as a GST-fusion protein (4). When fused to the N-terminus of another protein enzymatic activity is usually retained. *S. japonicum* GST is a protein with a M_r of 26,000. The crystal structure of the recombinant protein has been resolved (5) and shown to undergo dimerization similar to that observed in nature (6–8). For generation of constructs for expression of GST-fusion proteins, the sequences coding for the protein of interest can be recombinantly inserted into commercial vectors such as the pGEX (GE Healthcare) series of plasmids.

Glutathione HiCap Matrix represents a state-of-the-art matrix for affinity purification of GST and GST-fusion proteins via their strong and specific binding to the tripeptide ligand glutathione, which is stably immobilized on the Glutathione HiCap Matrix support. Glutathione HiCap Matrix and Glutathione HiCap Cartridge have an increased binding capacity of up to 50%, providing improved performance and highly reproducible results.

The simple bind-wash-elute workflow of glutathione affinity purification is shown in the flowchart on page 8. Figure 1 shows a typical result for purification of GST-tagged proteins using Glutathione HiCap Matrix for GST-DHFR expressed in *E. coli*.


Bind
GST
GSH
Target protein
Glutathione
HiCap Matrix
GSH: Reduced Glutathione
Wash
Contaminating proteins
Elute
Reduced
Glutathione
Applications
Endoproteolytic
cleavage
Target
protein
Immunological detection
(GST-tag Antibody)
GST pull-down assays
Activity-based assays

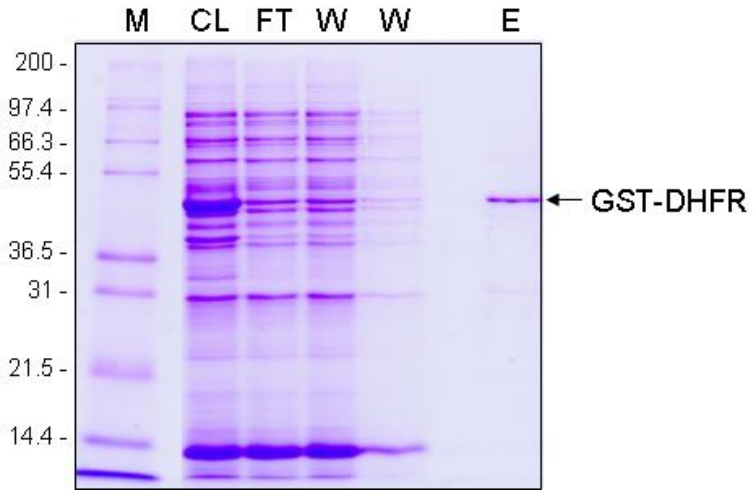


Figure 1. Purification of GST-tagged murine DHFR (dihydrofolate reductase) from *E. coli* BL21(DE3) under native conditions. Coomassie® stained SDS gel. **FT**: Flow-through fraction; **CL**: Cleared cell lysate; **E**: Elution fraction; **M**: Markers; **W**: Wash fractions.

In addition to its use for affinity purification, the GST tag is frequently utilized in so-called pull-down experiments to investigate protein:protein interactions. The pull-down assay is an in vitro method used to determine physical interaction between two or more proteins. Pull-down assays are useful for both confirming the existence of a protein:protein interaction predicted by other research techniques (e.g., co-immunoprecipitation) and as an initial screening assay for identifying previously unknown protein:protein interactions. The minimum requirement for a pull-down assay is the availability of a purified and tagged protein (the bait) which will be used to capture and 'pull down' a protein-binding partner (the prey). The Glutathione HiCap Matrix is highly suitable for GST pull-down experiments.

Purification scales and downstream applications

Protocols are provided in this handbook for lysis of *E. coli*, insect, and mammalian cells. The purification scale is dependent on the amount of protein in the preparation. The column size and total binding capacity should be chosen to approximately match the amount of protein to be purified (Table 1) to save resin and to suppress binding of unwanted proteins. Very few nontagged proteins may be retained on the resin when nearly all available glutathione binding sites are occupied by the target protein. If too much matrix is used, other proteins may bind nonspecifically to unoccupied sites and elute as contaminants.

Glutathione HiCap Matrix is suitable for batch purification, gravity-assisted chromatography, and packed-bed column chromatography (cartridges or custom-packed columns), as well as for high-throughput applications in vacuum- (9) or centrifugation-based protocols.

Glutathione HiCap Cartridges are pre-filled with 1 ml or 5 ml Glutathione HiCap Matrix and are ready to use for purification of GST-tagged proteins using a syringe, peristaltic pump, or automated liquid chromatography systems (such as ÄKTA, ÄKTAdesign™, or BioLogic workstation). The extremely high mechanical stability and the excellent flow characteristics of the Glutathione HiCap Matrix make it highly suited for both gravity flow-assisted and automated systems and provide high dynamic binding capacity. Nevertheless, optimal recovery of GST-tagged proteins is obtained at low flow rates during binding and highest protein concentration in the elution fraction will be achieved with low flow rate during protein elution (see Table 1). The outstanding stability of the matrix also means that Glutathione HiCap Matrix is well suited for GST pull-down applications, where the beads are subjected to repeated high *g*-force centrifugation steps.

Table 1. Characteristics of Glutathione HiCap Matrix and Glutathione HiCap Cartridges

	Glutathione HiCap Matrix	**1 ml Glutathione HiCap Cartridge**	**5 ml Glutathione HiCap Cartridge**
Support	Superflow (highly cross-linked 6% agarose)		
Suspension (slurry) concentration	50%		
Bead diameter	60–160 µm (mean: 90 µm)		
Ligand	Glutathione (Glu-Cys-Gly tripeptide)		
Column dimensions (mm)	–	6.7 x 28.0	14.7 x 29.8
Maximum pressure*	10 bar, 1.0 MPa	5 bar, 0.5 MPa	5 bar, 0.5 MPa
Typical back pressure (aqueous buffer, 10% glycerol, at room temperature)		1.0 bar, 0.1 MPa (1 ml/min)	2.0 bar, 0.2 MPa (5 ml/min)
Recommended flow rate†		0.25–1 ml/min (45–155 cm/h)	1.25–5 ml/min (45–155 cm/h)
Maximum flow rate‡	1560 cm/h	10 ml/min (1560 cm/h)	40 ml/min (1560 cm/h)

	Glutathione HiCap Matrix	1 ml Glutathione HiCap Cartridge	5 ml Glutathione HiCap Cartridge
Column connections	See Table 3, page 14		
Cartridge connectors	1/16" (inlet); M6 (outlet)		
Cartridge body material	Polypropylene		
System compatibility	Automated chromatography systems (e.g., ÄKTA, FPLC, BioLogic, Profinia)		
pH stability	3–12		
Chemical stability	All commonly used aqueous buffers; avoid strong oxidizing reagents and high temperatures; resistant to short exposure to denaturants (e.g., 6 M GuHCl), organic solvents (e.g., 70% ethanol), 1% (w/v) SDS, and aqueous solutions for cleaning-in-place (e.g., 0.1 M NaOH, 0.1 M HCl)		
Binding capacity[§]	Up to 20 mg/ml	Up to 20 mg	Up to 100 mg
Storage conditions	Store Glutathione HiCap Matrix and Cartridges in 20% (v/v) ethanol at 2–8°C		

* The maximum pressure usable with the matrix itself is 10 bar. However, stability of the cartridges is only guaranteed up to 5 bar.

† Recommended flow rate refers to the binding and elution steps; equilibration, wash and cleaning may be performed at higher flow rate (up to 2 ml/min, 310 cm/h, 1 ml cartridge).

‡ High flow rates may lead to reduced recovery of GST-tagged proteins.

§ Please note that the actual binding capacity is protein-dependent.

Cartridge connections

Glutathione HiCap Cartridges can be used for purification of proteins in a manual procedure (using a syringe) or an automated procedure using a chromatography system (such as the ÄKTAdesign or FPLC System). The cartridge inlet and outlet dimensions and required connectors and adapters for manual and automated procedures are detailed in Table 2.

Table 2. Connections and required connectors for Glutathione HiCap Cartridges

	Inlet	Outlet
Glutathione HiCap Cartridge connections	1/16″ female	M6 male (FPLC)
Adapters for manual procedure using a syringe	1/16″ male/luer female (e.g., GE Healthcare cat. no. 18-1112-51)	
Connector for automated procedure (ÄKTAdesign 1/16″ connectors	No adapter required	Union M6 female/1/16'' female (e.g., GE Healthcare cat. no. 18-1123-94)
Connector for automated procedure (M6 fittings, [FPLC])	Union M6 female/ 1/16″ male (e.g., GE Healthcare cat. no. 18-3858- 01)	SRTC-2, M6 female (0.5 mm i.d.) (e.g., GE Healthcare cat. no. 18-3856-01)

Visualization of GST-tagged protein

GST-tag Antibody allows immunologic applications such as western blot, enzyme-linked immunosorbent assay (ELISA), and immunoprecipitation. The antibody is highly sensitive and allows detection of amounts of GST protein as low as 0.5 ng (Figure 2, Table 3).

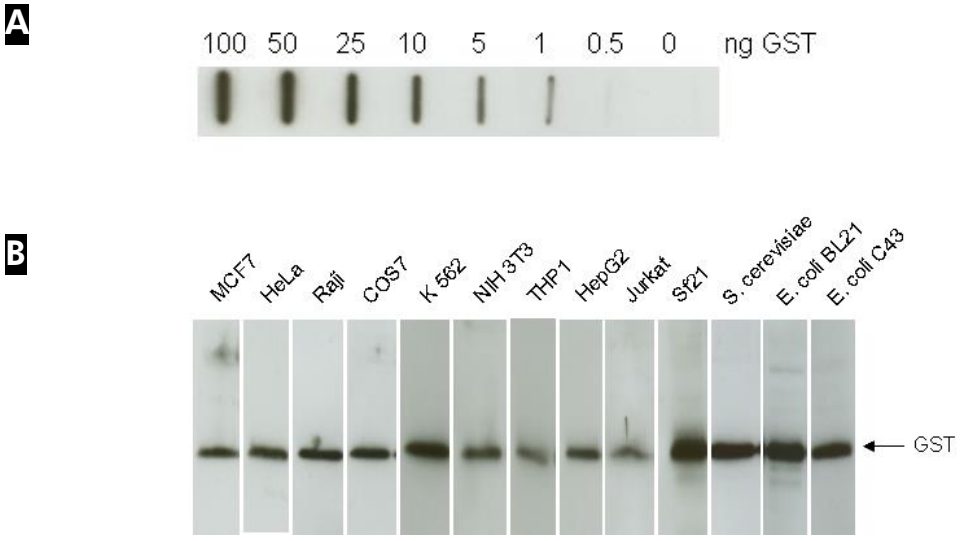


Figure 2. Sensitivity and specificity of GST-tag Antibody. **A** Slot blot. The indicated amounts of purified GST in denaturing buffer protein were slot blotted onto nitrocellulose membrane, probed with GST-tag Antibody at 1:1000 dilution, and detected using chemiluminescence (1 minute exposure). As little as 0.5 ng could be detected under these conditions. **B** Western blot. 100 ng of GST was mixed with 50 µg of the lysate derived from the indicated cells, separated by SDS-PAGE, and blotted onto nitrocellulose membrane. The membrane was probed with GST-tag Antibody at 1:1000 dilution and detected using chemiluminescence (1 minute exposure).

Table 3. Characteristics of QIAGEN's GST-tag Antibody

Feature	GST-tag Antibody (Mouse monoclonal antibody)
Detects	*S. japonicum* Glutathione S-Transferase (pGEX)*
Isotype	IgG2a
Applications	Western blot, ELISA, immunoprecipitation
Sensitivity†	Dot/slot blots: ≤1 ng Western blot: ≤0.5 ng
Specificity	No cross-reactivity with mammalian, yeast, insect, and *E. coli* cell proteins observed in western blot (100 ng GST in 50 µg cell lysate, see Figure 2)
Blocking conditions‡	5% (w/v) skim milk in TBS (recommended) or 5 % (5/v) alkal□-soluble casein in TBS
Working concentration	Western blot: 0.1–0.2 µg/ml (1:500 to 1:1000 dilution of a reconstituted 0.1 µg antibody per µl dH_2O stock solution) ELISA: 0.05 µg/ml Immunoprecipitation: 1–10 µg/ml
Secondary antibody	Anti-mouse IgG conjugated to a reporter (e.g., horseradish peroxidase [HRP], alkaline phosphatase [AP])
Form	Lyophilized; contains PBS with PEG, sucrose, and sodium azide (0.08% [w/v] concentration when diluted in 10 µl H_2O per µg)
Stability	At least 12 months (lyophilized, –15 to –30°C)
Storage (reconstituted)	Long-term: –80°C, 12 months Short-term: 2–8°C, 3 months

* Does not interact with mammalian GST.

† Sensitivity may vary with different GST-fusion proteins.

‡ Blocking conditions tested were suitable for chemiluminescent detection.

Removal of GST-tag using Factor Xa cleavage site

Following purification, it may be desirable to remove the GST moiety from the protein of interest for further investigation. Most vectors for expression of GST-fusion proteins, such as the pGEX plasmid series, encode an endoprotease site for GST cleavage. Endoproteolytic cleavage may also be performed on-column (10). A protocol for Factor Xa endoprotease cleavage is included in this handbook. Factor Xa allows separation of the protein of interest from GST without leaving any vector-encoded amino acids. Figure 3 shows the cleavage of the 35 amino acids V3 domain of HIV1-gp120 from its GST fusion.

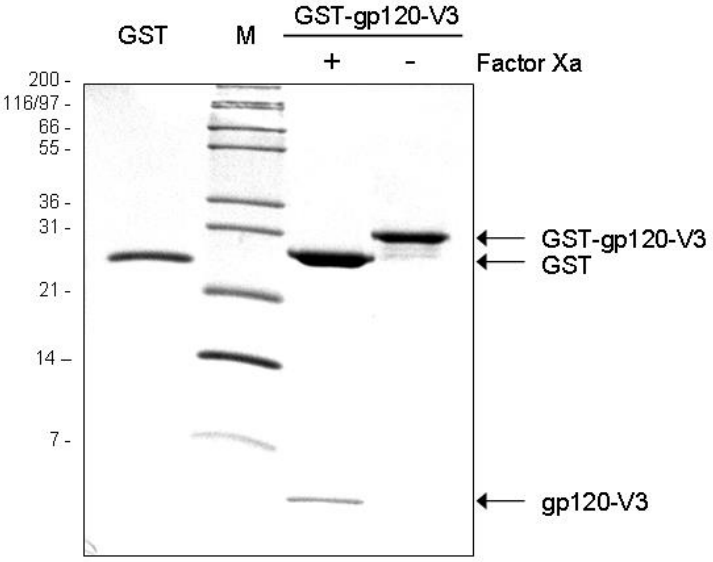


Figure 3. Endoproteolytic cleavage of the GST moiety from the fusion target protein gp120-V3 using Factor Xa. Coomassie-stained SDS-PAGE of the fusion protein GST-gp120-V3 in the absence and after digestion with Factor Xa (Purified GST was loaded as a control in lane 1). The 35 amino acids V3 domain of gp120 migrates at approximately 3 kDa.
M: Markers.

Optimization of protein purification

In cases where the quality or recovery of the target protein is not sufficient, for example, due to solubility issues or unwanted co-eluting proteins, there are several measures that can be taken to improve purification of GST-tagged proteins. For a detailed description of optimization options see Appendix B, page 45.

Protocol: Growth of *E. coli* Cells (200 ml)

E. coli cells may be grown in regular LB medium containing an appropriate antibiotic and induced for protein expression by addition of IPTG. Alternatively, cells may be grown unattended in autoinduction medium (see reference 11). Typical autoinduction media contain two carbon sources, glucose and lactose. Glucose will be metabolized first by *E. coli* (catabolite repression) and after it has been used up cells will switch their metabolism to lactose consumption thereby slowly inducing protein expression from Lac-regulated promoters (e.g., T7 system, T5 system, Lac, LacUV, trc).

Autoinduction usually results in higher biomass and protein expression levels.

Reagents to be supplied by user

- LB or autoinduction medium
- Antibiotics
- IPTG

Media compositions are provided in Appendix A on page 42.

IPTG induction

Procedure

1. **Inoculate 25 ml of LB medium containing the appropriate antibiotic(s) in a 100 ml flask. Grow the culture at 30°C overnight at 150 rpm.**

   Precultures should not grow too high and should not reach the static growth phase after overnight incubation.

2. **Inoculate 200 ml of prewarmed medium in a 2 l flask containing the appropriate antibiotic(s) to a starting OD_{600} of 0.05–0.1 using the preculture.**

   Determine the OD_{600} of the preculture and transfer the suitable volume to the main culture to reach the starting OD.

3. **Incubate at 18–37°C with shaking at approx. 100 rpm until OD_{600} of 0.6–1.0 has been reached.**

   Optional: Take a 0.5 ml sample immediately before induction.

   This sample is the noninduced control. Pellet cells and resuspend them in 25 µl 2x SDS-PAGE sample buffer. Freeze and store the sample at –15 to –30°C until SDS-PAGE analysis.

4. **Induce expression by adding IPTG to a final concentration of 1 mM.**

5. **Incubate the cultures for an additional 2–4 hours.**

   Optional: Collect a second 0.5 ml sample**.**

   This sample is the induced control. Pellet cells and resuspend them in 50 µl 2x SDS-PAGE sample buffer. Freeze and store the sample at –15 to –30°C until SDS-PAGE analysis.

6. **Harvest the cells by centrifugation at 4,000 x *g* for 20 min.**
7. **Freeze and store cell pellet at –15 to –30°C.**

Autoinduction

Procedure

1. **Inoculate 25 ml of LB medium containing the appropriate antibiotic(s) in a 100 ml flask. Grow the culture at 30°C overnight at 100 rpm.**

   Precultures should not grow too high and should not reach the static growth phase after overnight incubation.

2. **Inoculate 200 ml of prewarmed autoinduction medium in a 2 l flask containing the appropriate antibiotic(s) to a starting OD_{600} of 0.1 using the preculture.**

   Determine the OD_{600} of the preculture and transfer the suitable volume to the main culture to reach the starting OD.

   Optional: Take a 0.5 ml sample.

   This sample is the noninduced control. Pellet cells and resuspend them in 25 µl 2x SDS-PAGE sample buffer. Freeze and store the sample at –15 to –30°C until SDS-PAGE analysis.

3. **Grow overnight (18–24 h) at 18–37°C with shaking at approx. 150 rpm.**

   Optional: Collect a second sample (0.1 ml culture volume).

   This sample is the induced control. Pellet cells and resuspend then in 250 µl 2x SDS-PAGE sample buffer. Freeze and store the sample at –15 to –30°C until SDS-PAGE analysis.

4. **Harvest the cells by centrifugation at 4,000 x *g* for 20 min.**
5. **Freeze and store cell pellet at –15 to –30°C.**

Protocol: Preparation of Cleared *E. coli* Cell Lysates

This protocol describes the generation of a cleared lysate from *E. coli* cytoplasm under native conditions. The protocol may be modified depending on initial results which may have revealed (partial) insolubility of the GST-tagged protein or contamination of the preparation with unwanted proteins.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- *E. coli* cell pellet
- Buffer PBS-L (lysis buffer)
- Benzonase® Nuclease (3 U per ml culture; Novagen, cat. no. 71206-3)
- 2x SDS-PAGE sample buffer

Buffer compositions are provided in Appendix A on page 42.

Important points before starting

- Freeze *E. coli* cell pellet at –15 to –30°C for at least 30 minutes to ensure quantitative lysis by lysozyme. If fresh cells are used, lyse cells by physical disruption (homogenizer, French pressure cell, sonication).

Procedure

1. **Thaw the cell pellet derived from 50 ml *E. coli* cell culture on ice for 15 min and resuspend pellet in 5 ml buffer PBS-L.**

   The amount of cells required depends on the expression level of the GST-tagged protein and the expression system used. The binding capacity of GST affinity resin is protein-dependent and normally lies by 10 mg/ml.

2. **Incubate on an end-over-end shaker at room temperature (15–25°C) for 30 min.**
3. **Centrifuge lysate at 15,000 x *g* for 20–30 min at 4°C to pellet the cellular debris. Save the supernatant.**

   A proportion of the cellular protein, including the GST-tagged protein, may remain insoluble and will be located in the pellet. For more complete recovery of the tagged protein, this material must be solubilized under denaturing conditions (up to 4 M urea can be used).

4. **Add 5 µl 2x SDS-PAGE sample buffer to 5 µl supernatant and store at –15 to –30°C for SDS-PAGE analysis.**

5. **Proceed to either the manual purification protocol (page 22) or the cartridge purification protocol (page 24).**

Protocol: Preparation of Insect and Mammalian Cell Lysates

Insect cells are often the eukaryotic expression system of choice, since they provide post-translational modifications. The following protocol can be used as a starting point for developing a protocol for purification of GST-tagged proteins expressed intracellularly in insect cells. However, further optimization may be necessary depending on the tagged target protein. Expressed protein levels are typically lower than those obtained in bacterial systems, and in general, smaller amounts of starting cell material are available. The estimated total protein content in insect cell is 20 mg per 10^7 cells. With recombinant protein expression levels ranging between 0.05% and 50%, the theoretical maximum protein yield is 10 μg – 10 mg per 10^7 cells.

Expression levels in mammalian cells are typically considerably lower than in insect cells. However, mammalian expression systems allow isolation of mammalian proteins with the natural post-translational modification and are therefore often the system of choice for functional investigations such as protein:protein interactions. GST pull-down experiments (see protocol on page 28) are an ideal assay system for such investigations. As an estimation of the possible content in mammalian cells, HeLa cells, for example, contain approximately 3 mg of total protein per 10^7 cells. With recombinant expression levels at 0.01 % to 1% of total protein, typical protein yields are 0.3 to 30 μg per 10^7 cells.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Cell pellet
- Buffer PBS-L (lysis buffer; replace Triton X-100 by NP-40 and omit lysozyme)
- Benzonase Nuclease (3 U per ml culture; Novagen cat. no. 71206-3)

Buffer compositions are provided in Appendix A on page 42.

Important points before starting

- Buffer PBS-L should be supplemented with 1 % Igepal® CA-630 (Nonidet P40) for lysis of insect or mammalian cells.

Procedure

1. **Wash the transfected cells with PBS and collect them by centrifugation for 5 min at 1000 x *g*.**
2. **Lyse the cells in Buffer PBS-L supplemented with1% Igepal CA-630 using 4 ml buffer per 1–2 x 10^7 cells. Incubate for 10 min on ice.**
3. **Centrifuge the lysate at 10,000 x *g* for 10 min at 4°C to pellet cellular debris. Save the cleared lysate (supernatant).**

   The supernatant should contain the GST-tagged protein. In case the GST-tagged protein is a membrane protein and expected to be localized in a membrane, refer to the *Ni-NTA Membrane Protein Kit Handbook* (12) for generation of a membrane fraction from insect cells and perform a screen for the most suitable detergent for solubilization of the membrane protein.

   Add 5 µl 2x SDS-PAGE sample buffer to 5 µl supernatant and store at –15 to –30°C for SDS-PAGE analysis.
4. **Proceed to either the manual purification protocol (page 22) or the cartridge purification protocol (page 24).**

Protocol: Manual Purification of GST-Tagged Proteins

The protocol provided ensures optimal results (high protein purity, good recovery) in most cases. In cases where purity and recovery are compromised, results may be improved by measures described in Appendix B, page 45. The protocol is suitable for purification of up to 10 mg of GST-tagged protein from cells showing an expression rate of 25 mg per liter culture volume. If expression rate is known to be lower or higher, adjust the resin or culture volume processed accordingly.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Cleared lysate from a 200 ml culture volume
- Empty columns (e.g., Polypropylene Columns 1 ml, QIAGEN cat. no. 34924)
- Buffer PBS-EW (equilibration/wash buffer)
- Buffer TNGT (elution buffer)

Buffer compositions are provided in Appendix A on page 42.

Important points before starting

- Buffer TNGT for elution contains 400 mM NaCl, please ensure compatibility with your sample.

Procedure

1. **Pour 1 ml of the 50% Glutathione HiCap Matrix slurry (500 µl bed volume [bv]) into an empty column.**

   Resuspend the resin contained in the bottle completely by inverting until homogenous slurry is obtained. Pipet the resin slurry immediately to ensure transfer of the desired resin bed volume.

2. **Equilibrate the settled resin with 10 column volumes of buffer PBS-EW (5 ml buffer for 500 µl bv).**
3. **Load the lysate to the equilibrated resin and collect the column flow-through.**

   Save flow-through for SDS-PAGE analysis.

4. **Wash twice with 2.5 ml buffer PBS-EW (5 bv); collect wash fractions for SDS-PAGE analysis.**

5. **Add 500 µl elution buffer TNGT (1 bv) and collect the eluate containing the GST-tagged protein (elution fraction 1).**

   Optional: Incubate the resin in elution buffer for 15 min before collection of the elution fraction; this can increase protein concentration of the elution fraction. Apply 500 µl elution buffer (1 bv) and immediately seal the column with the bottom outlet cap and incubate for 15 minutes at room temperature (15–25°C); open the column and collect the eluate containing the GST-tagged protein (elution fraction 1).

6. **Add 500 µl elution buffer TNGT (1 bv) and collect the eluate (elution fraction 2).**

7. **Repeat step 6 twice or until GST-tagged protein has been eluted completely.**

   The number of elution steps required to completely elute the GST-tagged protein from the Glutathione HiCap Matrix may vary with the nature and amount of bound protein.

8. **Analyze fractions by SDS-PAGE and/or Bradford or other suitable protein assay.**

Protocol: Purification of GST-Tagged Proteins Using Glutathione HiCap Cartridges

If larger amounts of protein are to be purified or if the purification will be performed using FPLC equipment, a Glutathione HiCap Cartridge should be used. The physical stability of Glutathione HiCap Matrix is optimal for column chromatography at higher pressures and flow rates. The protocol provided gives good results (high protein purity, good recovery) in most cases. In cases where purity and recovery is compromised, results may be improved by measures described in Appendix B, page 45. The protocol is suitable for purification of up to 20 mg (1 ml cartridge) or 100 mg of GST-tagged protein (5 ml cartridge) from cells showing an expression rate of 25 mg per liter culture volume. If expression rate is known to be lower or higher, adjust the resin or culture volume processed accordingly.

Things to do before starting

- Filter cleared lysates using a 0.45 μm filter.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Cleared lysate from a 400 ml culture volume
- Buffer PBS-EW (equilibration/wash buffer)
- Buffer TNGT (elution buffer)

Buffer compositions are provided in Appendix A on page 42.

Procedure

1. **Equilibrate the resin with 10 column volumes of buffer PBS-EW at 310 cm/h.**

   1 ml Glutathione HiCap Cartridge: 10 ml; 2 ml/min.

   5 ml Glutathione HiCap Cartridge: 50 ml; 10 ml/min.

2. **Load the lysate to the equilibrated resin at 45–155 cm/h and collect the cartridge flow-through.**

   1 ml Glutathione HiCap Cartridge: 0.25–1 ml/min.

   5 ml Glutathione HiCap Cartridge: 1.25–5 ml/min.

   Save flow-through fraction for SDS-PAGE analysis.

3. **Wash with 10 column volume of buffer PBS-EW at 310 cm/h; collect wash fractions for SDS-PAGE analysis.**

   1 ml Glutathione HiCap Cartridge: 10 ml; 2 ml/min.

   5 ml Glutathione HiCap Cartridge: 50 ml; 10 ml/min.

4. **Elute the protein with 10 column volumes of elution buffer TNGT at 45–155 cm/h. Collect the elution fractions in 10 tubes and analyze by SDS-PAGE.**

   1 ml Glutathione HiCap Cartridge: 0.25–1 ml/min.

   5 ml Glutathione HiCap Cartridge: 1.25–5 ml/min.

   A low flow rate can increase the concentration of the GST-tagged protein in the elution fractions.

Protocol: Immunodetection of GST-Tagged Proteins

This protocol is used for chemiluminescent detection of GST-tagged proteins on Western, dot, or slot blots.

Alternatively, chromogenic detection may be performed in step 8. Either AP- or HRP-conjugated anti-mouse IgG may be used.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Western blot or dot blot
- TBS (10 mM Tris·Cl; 150 mM NaCl, pH 7.5)
- TBS-Tween®/Triton (20 mM Tris·Cl; 500 mM NaCl; 0.05% (v/v) Tween; 0.2% (v/v) Triton, pH 7.5)
- Blocking buffer (5% skim milk powder in TBS)
- GST-tag Antibody stock solution: Dissolve the lyophilized GST-tag Antibody in 1000 µl water at room temperature (15–25°C). The reconstituted solution contains 100 µg/ml GST-tag Antibody in PBS with PEG, sucrose, and sodium azide (0.08% [w/v]).
- Anti-mouse secondary antibody conjugate
- Secondary antibody dilution buffer (5% skim milk powder in TBS)

The solutions required depend on the detection method employed.

Procedure

1. **Wash membrane twice for 10 min each time with TBS buffer at room temperature (15–25°C).**
2. **Incubate for 1 h in blocking buffer at room temperature.**
   5% skim milk powder (w/v) in TBS buffer is used for blocking.
3. **Wash membrane twice for 10 min each time in TBS-Tween/Triton buffer at room temperature (15–25°C).**
4. **Wash membrane for 10 min with TBS buffer at room temperature (15–25°C).**
5. **Incubate with GST-tag Antibody (1/500–1/1000 dilution of antibody stock solution in blocking buffer) at room temperature (15–25°C) for 1 h.**
   Membrane can be sealed in plastic bags. Use 5% skim milk powder (w/v) in TBS buffer for this blocking step when performing chemiluminescent detection.

6. **Wash twice for 10 min each time in TBS-Tween/Triton buffer at room temperature (15–25°C).**
7. **Wash for 10 min in TBS buffer at room temperature (15–25°C).**
8. **Incubate with secondary antibody solution in blocking buffer for 1 h at room temperature (15–25°C).**

   Either AP- or HRP-conjugated anti-mouse IgG may be used. Use 5% skim milk in TBS for incubation with secondary antibody when performing chemiluminescent detection. When performing chromogenic detection, follow the manufacturer's recommendations. Use the lowest recommended concentration to avoid false signals.
9. **Wash 4 times for 10 min each time in TBS-Tween/Triton buffer at room temperature.**
10. **Perform the chemiluminescent detection reaction and expose to x-ray film according to the manufacturer's recommendations.**

Protocol: GST Pull-Down Assay

This protocol is designed to conduct protein:protein interaction analysis using a GST-tagged protein immobilized to Glutathione HiCap Matrix and probing this 'bait' to catch an interacting protein — the prey. There are two general uses of the GST pull-down experiment. One is to identify novel interactions between a fusion protein and unknown proteins; another is to identify interactions between the fusion protein and a known protein that is a suspected interactor. The two experiments are designed and executed slightly different. See references 13 and 14 for more information.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Coomassie staining solution
- **Optional**: Silver staining solution
- Buffer TN1 (Tris/NaCl-based GST pull-down binding and wash buffer)
- Buffer TN2 (Tris/NaCl-based GST pull-down elution buffer) or 2x SDS-PAGE buffer for protein elution
- GST (control) as well as GST-fusion bait protein in buffer TN1 (~25 µg each)
- Prey protein sample in buffer TN1 (amount of purified known protein: equimolar to bait GST-tagged protein; amount of cell lysate: equivalence to 1 x 10^6 to 1 x 10^7 cultured cells is a good starting point)

Buffer compositions are provided in Appendix A on page 42.

Important points before starting

- Keep buffers and protein solutions cold during all steps of the procedure

Things to do before starting

- Express the GST-fusion bait protein
- Express GST nonfusion protein as a control
- Prepare prey sample (e.g., labeling with S^{35} using the EasyXpress® Protein Synthesis Kit)

Procedure

1. **Equilibrate 3 portions of 50 µl Glutathione HiCap Matrix (50% slurry) using buffer TN1.**

   Resuspend Glutathione HiCap Matrix completely. Transfer 50 µl into three 2 ml reaction tubes and add 1 ml buffer TN1. Mix by inverting the tube. Centrifuge Glutathione HiCap Matrix at 1000 x *g* for 2 min. Remove buffer and resuspend in 50 µl buffer TN1.

2. **Mix the GST-tagged (bait) protein and the GST control protein, respectively, with the prey protein sample and equilibrated Glutathione HiCap Matrix according to Table 4.**

Table 4. GST pull-down assay and controls pipetting scheme

Reaction component	Tube 1	Tube 2	Tube 3
GST-tagged protein (bait)	25 µg		–
GST nontagged control	–	25 µg	–
Prey protein sample* (25 µg) or cell lysate*	Add*	Add*	Add*
Glutathione HiCap Matrix from step 1 (in Buffer TN1)	50 µl	50 µl	50 µl
Buffer TN1	To an equal final volume (0.5–1 ml)		

* Must not contain GST-tagged protein; equal amounts should be used for all three reactions; lysates may be pre-cleared if detection uses radioactivity (see Lysate pre-clearing option, below).

Tube 2 acts as a control to check that the interacting (prey) protein does not bind to the GST itself; tube 3 acts as a control to check that the interaction (prey) protein does not bind nonspecifically to the Glutathione HiCap Matrix. The assay may be scaled down to use <25 µg of bait and prey proteins.

Lysate pre-clearing option: If working with cell lysate as the source of prey sample, the lysate for tubes 1 and 2 may be pre-incubated with 100 µl of equilibrated Glutathione HiCap Matrix. This step is designed to pre-clear proteins from the lysate that may interact nonspecifically with the GST moiety or with the beads alone. This is especially useful to reduce the background if radioactively labeled samples are used with x-ray film exposition. Tube 3 does not need to be set up in this case. Incubate for 2 hours at 2–8°C with end-over-end mixing and save supernatant after centrifugation at 1,000 x *g* for 2 min. Equal amount of the precleared lysate is then added to tubes 1 and 2 according to Table 4.

3. **Mix the tubes by inverting and incubate for 2 hours or overnight at 2–8°C with end-over-end mixing.**
4. **Centrifuge reactions corresponding to tubes 1–3 at 1000 x *g* for 2 min at 2–8°C and remove the supernatant.**

   Save the supernatants in fresh reaction tubes (for control analysis using SDS-PAGE).
5. **Wash the beads 3 times with buffer TN1.**

   Discard washes (supernatants).
6. **Elute the GST-fusion protein and all proteins bound to it by adding 50 µl buffer TN2 to the beads. Gently mix and incubate for 10 min.**

   Alternatively, beads can be mixed with 50 µl 2x SDS-PAGE sample buffer and boiled at 95°C for 3 min prior to gel loading. In this case, it is important to include the beads-only control (tube 3) as proteins bound nonspecifically to the beads can appear as bound to the fusion protein even in comparison to GST alone (tube 2).
7. **Centrifuge reactions corresponding to tubes 1–3 at 1000 x *g* for 2 min at 2–8°C and collect the supernatant containing the protein complexes to be analyzed.**
8. **Analyze the proteins by SDS-PAGE.**

   If the goal is to detect the radioactively (usually ^{35}S-) labeled proteins associated with the GST-fusion protein after SDS-PAGE, the gel should be dried and exposed to an x-ray film.

   If the goal is to detect specific partners, after SDS-PAGE the proteins should be transferred to a membrane for western blot analysis.

   If the goal is to determine the size and abundance of proteins associated with the GST-fusion protein from a non-radioactive sample subsequent to SDS-PAGE, the gel should be stained using Coomassie or silver nitrate.

Protocol: Factor Xa-mediated Endoproteolytic Cleavage of GST from GST-Fusion Proteins

Many vectors suitable for expression of GST-tagged proteins (e.g., the pGEX series) provide a recognition site for endoproteases for cleavage of GST-fusion proteins for recovery of the native target protein. Here, we provide a protocol for optimization of cleavage by Factor Xa which allows recovery of the target protein without any vector-encoded amino acids. Other endoproteases (e.g., Thrombin, Enterokinase, PreScission™) may be used if appropriate and protocols are provided by the manufacturers.

Factor Xa Protease is a site-specific endoprotease that preferentially cleaves the C-terminal peptide bond of the recognition sequence Ile-Glu-Gly-Arg. Factor Xa Protease consists of two polypeptides linked by a disulfide bond. The optimal cleavage conditions must be determined individually for each protein to be cleaved. Accessibility of the cleavage site, the adjacent amino acid sequence, and the degree of protein aggregation all affect cleavage efficiency. Optimization of Factor Xa Protease concentration, temperature (4–37°C), and incubation time (2–16 h) is recommended. Note that excess Factor Xa Protease may result in nonspecific proteolysis at secondary sites. Therefore, optimal enzyme specificity is achieved using the lowest amount of protease necessary to achieve complete cleavage.

As an approximation, one unit cleaves >95% of 50 µg of a test-fusion protein at 20°C in 16 hours. Nevertheless, an optimization of the cleavage conditions should be performed in small-scale reactions using the following protocol as a starting point. We recommend using 20 mM Tris·Cl, pH 6.5–7.5; 50 mM NaCl; 1 mM $CaCl_2$ (buffer TNC) as the reaction buffer.

Equipment and regents to be provided by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Factor Xa endoprotease (400 Units, 2 U/µl; QIAGEN, cat. no. 33223)
- Factor Xa removal resin (QIAGEN, cat. no. 33213)
- Disposable column (bottom closure cap required for Factor Xa cleavage)
- Purified GST-tagged protein (purified or Glutathione HiCap Matrix-bound) containing a Factor Xa recognition sequence; provide in buffer TNC
- Buffers TNC (Factor Xa reaction buffer) and PBS-EW

Buffer compositions are provided in Appendix A on page 42.

Things to do before starting

- If the cleavage reaction will be carried out with eluted GST-tagged protein in solution, buffer conditions must be adjusted to allow Factor Xa cleavage, e.g., by dialysis versus Factor Xa cleavage buffer TNC.

In-solution cleavage

Procedure

1. **Prepare 4 solutions each containing 10 µg of the protein to be cleaved in TNC buffer. The solutions should have a protein concentration of at least 0.25 µg/µl.**

   Since Factor Xa Protease is sensitive to various buffer constituents, we recommend that the protein to be cleaved is prepared in TNC buffer before cleavage. If your individual protein requires other specific buffering conditions, please see Appendix D, page 49 for the compatibility of some commonly used buffer components with Factor Xa Protease.

2. **Prepare 3 serial dilutions of Factor Xa Protease in 1x reaction buffer with concentrations of 1.0, 0.2, and 0.05 Units per µl.**

   It is important to mix the dilutions completely before use to avoid localized differences in enzyme concentration. Diluted protease should be used immediately after preparation.

3. **Add 1 µl of each Factor Xa Protease dilution to one solution of the protein to be cleaved, and adjust the reaction volume to 40 µl using buffer TNC. Adjust the volume of the fourth protein solution to 40 µl using buffer TNC. This sample will serve as a negative control to monitor the progress of cleavage.**

Table 5. In-solution cleavage pipetting scheme

Component	Volume/reaction	Final concentration
10 µg GST-fusion protein	Variable	0.25 µg/µl
Factor Xa protease dilutions	1 µl	1.0, 0.2, and 0.05 U/40 µl
TNC buffer	Adjust to 40 µl	

4. **Incubate the reactions at room temperature (15–25°C). Take an 8 µl aliquot from each reaction after 3, 6, 9, and 16 h. Add 2 µl 5x SDS-PAGE sample buffer to each aliquot and mix thoroughly.**

   It is important to mix the aliquots with SDS-PAGE sample buffer immediately to completely quench Factor Xa Protease activity.

5. **Analyze the efficiency of cleavage in each sample by SDS-PAGE.**

   Factor Xa-mediated cleavage may be optimized further (see Appendix D, page 49). See below for a protocol to remove Factor Xa Protease.

Scale-up

Once the optimal cleavage conditions have been determined, the reaction can be scaled up proportionally. Following the above protocol, 1 mg recombinant protein would be digested in a total volume of 4 ml. If there is a need to reduce the total reaction volume, perform small-scale experiments in which the reaction volume is varied while the protease:recombinant protein ratio and incubation conditions are kept constant.

Removal of GST-cleavage product and of undigested GST-fusion protein after Factor Xa treatment

This step is not required if the GST-fusion protein has been cleaved using the on-column protocol.

1. **If required, adjust the pH of the cleavage reaction mixture to 7.5.**

   If the cleavage reaction had been performed at pH 6.5 adjustment to 7.5 can be achieved by the addition of ~1/100 volume of 1M Tris·Cl, pH 8.0. Factor Xa Protease cleavage and removal are optimal at pH between 6.5 and 7.5.

2. **Calculate the required amount of Glutathione HiCap Matrix needed to capture the GST cleavage product.**

   A bed volume of 1 ml (2 ml slurry) is sufficient to bind 5–10 mg GST-tagged protein. For optimal performance, the binding capacity of the Glutathione Superflow used for removal should match the total amount of GST-tagged protein that was subjected to Factor Xa Protease cleavage.

3. **Resuspend the Glutathione HiCap Matrix completely by gently inverting the bottle and then immediately transfer the required amount of slurry into a disposable column.**
4. **Equilibrate with 10 bed volumes of buffer 20 mM Tris·HCl, pH 7.2, 50 mM NaCl.**
5. **Transfer the pH-adjusted reaction mixture to the equilibrated resin and collect the flow-through.**
6. **Apply one bed volume and collect together with the flow-through fraction from step 6.**

   This fraction will contain the majority of the processed, i.e., untagged protein of interest (plus the Factor Xa Protease). The column may be washed further to recover the residual processed protein of interest.

On-column cleavage

Procedure

This protocol is designed for cleavage of the fusion protein from its GST tag while immobilized on Glutathione HiCap Matrix (1 ml bed volume, e.g., using a 1 ml Glutathione HiCap Cartridge). The scale may be adjusted proportionally.

1. **Equilibrate/wash the resin (with the GST-tagged protein bound) using 10 ml (10 bed volumes) of buffer TNC.**

   This step serves to provide optimal reaction conditions for Factor Xa cleavage. Factor Xa activity is reduced in the presence of phosphate.

   Gravity-assisted column: Let the resin drain completely by gravity flow until no more buffer stands above the resin.

2. **Prepare Factor Xa Protease cleavage solution: Mix 80 µl (160 units) of protease with 920 µl of buffer TNC by gently inverting the vessel.**

   This amount of Factor Xa Protease is sufficient to cleave approximately 10 mg of GST-fusion protein on the column.

3. **Load the Factor Xa cleavage solution onto the column.**

   Glutathione HiCap Cartridge: Load 1 ml of the cleavage solution onto the cartridge using a syringe.

   Glutathione HiCap Matrix gravity-assisted flow column: Pipet 1 ml of the cleavage solution onto the resin and let the column drain completely by gravity flow. Do not force additional liquid out of the resin by applying pressure.

4. **Close the both the inlet and outlet of the cartridge (column).**

   Glutathione HiCap Cartridge: use the plugs provided.

   Glutathione HiCap Matrix gravity-assisted flow column: close column outlet using the cap provided, seal inlet opening with foil (e.g., Parafilm®), and incubate in an upright position.

5. **Incubate the column at room temperature (15–25°C) for 2–16 h.**
6. **Apply one bed volume of buffer 20 mM Tris·HCl, pH 7.2 and 50 mM NaCl and collect together with the flow-through fraction from step 5.**

   This fraction contains both the target protein that has been cleaved off from GST, as well as the Factor Xa protease. See below for a protocol to remove Factor Xa Protease.

Removal of Factor Xa Protease

After protease digestion, Factor Xa Protease can be removed by affinity chromatography using Xa Removal Resin. The Xa Removal Resin binds the protease in the reaction mixture while the cleaved recombinant protein remains

in solution. After the resin is pelleted by centrifugation, the cleaved recombinant protein is recovered in the supernatant.

Procedure

1. **Calculate the required amount of Xa Removal Resin necessary to capture the Factor Xa Protease present in the cleavage reaction.**

   A bed volume of 50 µl (100 µl slurry) is sufficient to bind 4 Units Factor Xa Protease enzyme in 1x reaction buffer. Use of slurry volumes of less than 25 µl is not recommended due to associated handling problems. If the recombinant protein requires a cleavage buffer other than the recommended 1x reaction buffer, consider that the protease capture step may be sensitive to the use of other buffers. Binding of Factor Xa Protease to the Xa Removal Resin is unaffected by increasing the pH to 7.5, the presence of 20–100 mM Tris·HCl, and up to 1% Triton X-100 or Nonidet P-40. High salt concentrations will reduce binding capacity. For example, increasing NaCl concentration from 50 mM to 500 mM will result in a 20–40% reduction in binding. The recommended buffer (20mM Tris·HCl, pH 7.2; 50 mM NaCl) supports high-efficiency cleavage and capture.

2. **Resuspend the Xa Removal Resin completely by gentle inversion and then immediately transfer the required amount of slurry into a centrifuge tube of appropriate size.**

   Note: The beads will quickly fall out of suspension. For transfer, use a wide-mouth pipet.

3. **Centrifuge the beads for 5 min at 1000 x *g* and discard the supernatant.**

4. **Resuspend the beads in 10 bed volumes of buffer 20 mM Tris·HCl, pH 7.2; 50 mM NaCl by gently mixing, centrifuge for 5 min at 1000 x *g*, and discard the supernatant.**

   Equilibration of the beads with buffer is necessary for maximum capture efficiency and prevents contamination of the cleaved recombinant protein with resin storage buffer. Use Xa Removal Resin immediately after equilibration.

5. **Add the cleavage reaction to the equilibrated resin. Mix gently to resuspend the resin and incubate for 10 min at room temperature (15–25°C). Rotate end-over-end or place the sealed tube on a roller-table to keep beads in suspension.**

   If the cleaved protein is temperature-sensitive, binding can be performed at 4°C without any loss of binding efficiency.

6. **Centrifuge the reaction at 1000 x *g* for 5 min to pellet the resin. Collect the supernatant which contains the cleaved protein. Factor Xa Protease remains bound to the resin.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
No or low protein expression	
a) Protein seems poorly expressed	Check that the protein is not found in the insoluble fraction. If so, refer to Appendix B, page 45 for optimization.
b) Culture conditions are incorrect	Use the same culture conditions and host cells to check for expression of GST protein alone encoded by a pGEX plasmid lacking an insert.
c) Protein is rapidly degraded	Perform a time course to check the kinetics of growth, expression, and degradation; shorten induction period. If degradation occurs after cell lysis, add protease inhibitors. Keep all samples and solution at 2–8°C during every step.
Protein purification	
a) Protein is (partly) in the insoluble fraction of the lysate	Refer to Appendix B, page 45 for optimization of lysis conditions.

	Comments and suggestions
b) Protein does not bind to Glutathione HiCap Matrix	Check flow rate and buffer conditions during binding step. The pH of the buffer should be between 6.5 and 8.0. GST may not be freely accessible. Try to partly destabilize the GST-fusion protein; evaluate addition of reducing reagents during cell lysis and binding (refer to Appendix B, page 45 for optimization). If reusing the resin check that it has been cleaned and regenerated correctly (see Appendix C, page 48). If in doubt, replace with fresh resin/cartridge.
c) Column is clogged	Cell debris in the sample may clog the column. Clean the column as outlined in Appendix C, page 48. Take care not to transfer any part of the cell debris pellet when generating the cleared lysate; filter the cleared lysate (0.45 μm filter) and/or increase centrifugation force to 30,000 x g (important if using packed columns and cartridges).
d) Protein poorly eluted	Increase glutathione concentration. Usually, GST-tagged proteins elute reliably at 50 mM glutathione. Increase pH up to a value of 9 in elution buffer. Increase ionic strength; ionic interaction may retain the protein on the resin; note that very hydrophobic proteins may precipitate under high-salt conditions. In such a case, try a nonionic detergent (e.g., 0.1 % [v/v] Triton X - 100) Increase incubation time of elution buffer on the resin before draining the eluate.

	Comments and suggestions
e) Protein preparation not pure	Protein may have become degraded. Include protease inhibitors in all steps/buffer used for purification. A prominent 65–70 kDa co-purifying band (probably DnaK from *E. coli*) can be efficiently removed by an additional wash step (ATP buffer, see Appendix B, page 45). Too much resin was used. If possible, match the total binding capacity approximately to the amount of GST-tagged protein present in your sample. Perform an additional purification step (e.g., ion exchange, gel filtration).
Detection using the GST-tag Antibody	
High background in western blot image	Inappropriate wash conditions or insufficient washing; evaluate the procedure and be sure to follow the recommended protocol. Improper blocking reagent and/or blocking time; use sufficient volume of 5% skim milk in TBS; use the lowest concentration of secondary antibody recommended by the manufacturer. Reduce development and/or x-ray film exposure time.

	Comments and suggestions
Factor Xa Protease cleavage	
a) No or incomplete cleavage	Insufficient Factor Xa Protease. Increase the amount of protease while keeping the recombinant protein concentration constant.
	Recombinant protein concentration too low. Increase protein concentration; a minimum of 0.25 µg/µl is recommended.
	Buffer component inhibits cleavage. Ensure that there are no buffer components that inhibit Factor Xa Protease (see Appendix D, page 49). Dialyze the GST-tagged protein against Buffer TNC to achieve optimal cleavage.
	Inappropriate incubation time and temperature. Increase temperature up to 37°C and prolong incubation time to 24 h.
b) Multiple bands observed on SDS gel following cleavage	Bands may derive from Factor Xa. If large amounts of Factor Xa Protease are used, two bands (~17–20 kDa and 28–30 kDa) may appear on the gel under reducing conditions. Run Factor Xa protease alone in an adjacent gel lane as a control.
	Protease cleaves within the recombinant protein. Check that no other Factor Xa recognition site is present in the recombinant protein. Reduce incubation temperature (room temperature [15–25°C] or 2–8°C) to minimize exposure of secondary cleavage sites.
	Reduce protease amount to exclude unspecific cleavage due to too high enzyme concentration.
	Adjust reaction conditions to obtain partial digestion which may result in selective scission at the desired Factor Xa Protease recognition site.

	Comments and suggestions
Factor Xa removal	
Factor Xa Protease not efficiently removed	Amount of removal resin too low. Check calculations for the amount of resin required (see protocol). Perform a second capture reaction. Buffer contains components which may affect the Factor Xa Protease binding reaction. Dialyze the cleavage reaction mixture against Buffer TNC and repeat the binding protocol (try to eliminate incompatible buffer components during the Factor Xa Protease cleavage reaction and repeat the cleavage and capture steps).
Removal of GST protein after Factor Xa cleavage	
GST binds insufficient or not at all to Glutathione HiCap Matrix	Binding conditions may not be correct. Check the pH and composition of reaction mixture. Be sure to equilibrate the resin prior to the capture step using a buffer with a pH value between 6.5 and 8.0; pH value is critical for efficient binding. Perform a second capture reaction.
GST pull-down assay	
a) Low signal	Interactor insufficiently present in the sample. Check for its presence if feasible. If the interactor is abundant in the probing sample, this may indicate that the binding conditions are not optimal. A change in salt and detergent type and/or concentration and an increase in time allowed for association may improve the result. The complex may be retained on the Glutathione HiCap Matrix due to inefficient elution. Elute by boiling with 2x SDS-PAGE sample buffer instead of reduced glutathione. If this is the problem, increase time for elution and/or concentration of reduced glutathione in Buffer TN2 up to 50 mM.

	Comments and suggestions
b) Nonspecific background	Unspecific binding of proteins from the probing lysate sample to either GST or to the Glutathione HiCap Matrix. Preclearing the lysate with GST or beads alone to minimize nonspecific interactions (see protocol). Decrease amount of lysate applied per reaction. Increase stringency of wash conditions.
c) Analysis reveals inconsistent band pattern on western blot	Determine whether all samples were incubated with the same amount of GST-fusion protein by reprobing the membrane with GST-tag Antibody. This will also help determine whether the fusion protein is undergoing degradation while incubated with the cell lysate.

Appendix A: Composition of Buffers

LB medium

10 g/l tryptone

5 g/l yeast extract

10 g/l NaCl

Autoclave; supplement with antibiotic under sterile conditions immediately before use and not before the medium has cooled down to 50°C.

Autoinduction medium

See reference 11 for the most suitable media.

PBS

50 mM NaH_2PO_4, pH 7.2

150 mM NaCl

PBS-L (PBS-based lysis buffer)

1 x PBS (50 mM NaH_2PO_4, 150 mM NaCl, pH 7.2)

Protease inhibitors (e.g., Roche Complete Cocktail, cat. no. 04693124001)

Benzonase (3 U per ml culture, Novagen (cat. no. 71206-3)

1 mM DTT

1 mM EDTA

1% (v/v) Triton X-100

1 mg/ml Lysozyme

For lysis of insect cells, replace Triton X-100 with 1% (v/v) Igepal CA-630 (Nonidet P40, NP-40) and omit lysozyme.

PBS-EW (PBS-based equilibration and wash buffer)

1 x PBS (50 mM NaH_2PO_4, 150 mM NaCl, pH 7.2)

1 mM DTT

1 mM EDTA

TNGT (elution buffer)

50 mM Tris pH 8.0

0.4 M NaCl

50 mM red. GSH (L-Glutathione reduced, e.g. Sigma, cat. no. G4251-5G)

0.1 % Triton-x-100

1 mM DTT

ATP buffer

50 mM Tris·HCl, pH 7.4

2 mM ATP

10 mM $MgSO_4$

TN1 (Tris/NaCl-based GST pull-down binding and wash buffer)

50 mM Tris·HCl, pH 8.0

200 mM NaCl*

1 mM EDTA*

1 % (v/v) Igepal CA-630 (Nonidet P-40)*

1 mM DTT*

10 mM $MgCl_2$*

Protease inhibitors as appropriate (e.g., Roche Complete Cocktail, see above)

* May be modified according to the specific requirements of the proteins analyzed.

TN2 (Tris/NaCl-based GST pull-down elution buffer)

50 mM Tris·HCl, pH 8.0

400 mM NaCl*

50 mM reduced glutathione

1 mM EDTA*

1 mM DTT*

Protease inhibitors as appropriate (e.g., Roche Complete cocktail, see above)

TNC (Factor Xa reaction buffer)

20 mM Tris·Cl, pH 7.5

50 mM NaCl

1 mM $CaCl_2$

Note: This buffer must not contain protease inhibitors. The pH may vary between 6.5 and 7.5 for optimal cleavage efficiency.

2x SDS-PAGE sample buffer

90 mM Tris·HCl, pH 6.8

20% (v/v) glycerol

2 % (w/v) SDS

0.02 % (w/v) bromophenol blue

0.1 M DTT

5x SDS-PAGE sample buffer

225 mM Tris·HCl, pH 6.8

50% (v/v) glycerol

5% (w/v) SDS

0.05% (w/v) bromophenol blue

0.25 mM DTT

* May be modified according to the specific requirements of the proteins analyzed.

Appendix B: Optimization of GST-Tagged Protein Purification

Improvement of recovery by partial destabilization

If the GST-tagged protein is not soluble under the recommended native buffer conditions (PBS-L, see protocols, pages 22 and 24) and is mostly or completely in the insoluble fraction, or if it does not bind to the Glutathione HiCap Matrix, you may try to destabilize conformation of the GST-tagged protein by adding the denaturant urea. Up to 4 M urea has been successfully applied in the lysis and binding step and is, in principle, compatible with GST binding to immobilized glutathione. The concentration of urea or other reagents that is compatible with the target fusion protein may vary and must be determined, since the activity and structure of several proteins may be reduced by chaotropes or detergents. Table 6 lists a selection of reagents and their highest concentrations that have been successfully used in glutathione affinity applications.

Table 6. Reagents compatible with glutathione affinity purification (may not be complete and concentrations may not be the compatible maxima)

Reagent	Concentration tested
Denaturants	
Urea	Up to 4 M
Guanidinium hydrochloride (GuHCl)	Up to 3 M
Detergents	
Triton X-100	Up to 2 % (v/v)
Tween 20	Up to 2 % (v/v)
SDS	Up to 0.03 % (w/v)
Cetyltrimethylammonium bromide (CTAB)	Up to 1 %
DDM	0.1 % (w/v; ~10x cmc)
CHAPS	1 % (w/v; ~10x cmc)
β-OG	5 % (w/v; ~10x cmc)
$C_{12}E_8$	0.05 % (w/v; ~10x cmc)
Brij 35	0.1 % (w/v; ~10x cmc)
NP-40	0.2 % (v/v; ~10x cmc)
Cholate	5 % (v/v; ~0.3x cmc)
Deoxycholate	2 % (w/v; ~0.2x cmc)
Reducing reagents	
DTT	Up to 10 mM
Salts	
NaCl	1 M

Improvement of purity by performing an additional wash step in the presence of ATP and $MgSO_4$

In some cases, a 65–70 kDa protein band from *E. coli* co-elutes with the target GST-fusion protein from Glutathione HiCap Matrix. This well-known contaminant is most likely the chaperone DnaK. If an additional wash step is performed in the presence of ATP and $MgSO_4$, this protein is reliably and specifically removed, leading to highly pure target fusion protein (Figure 4).

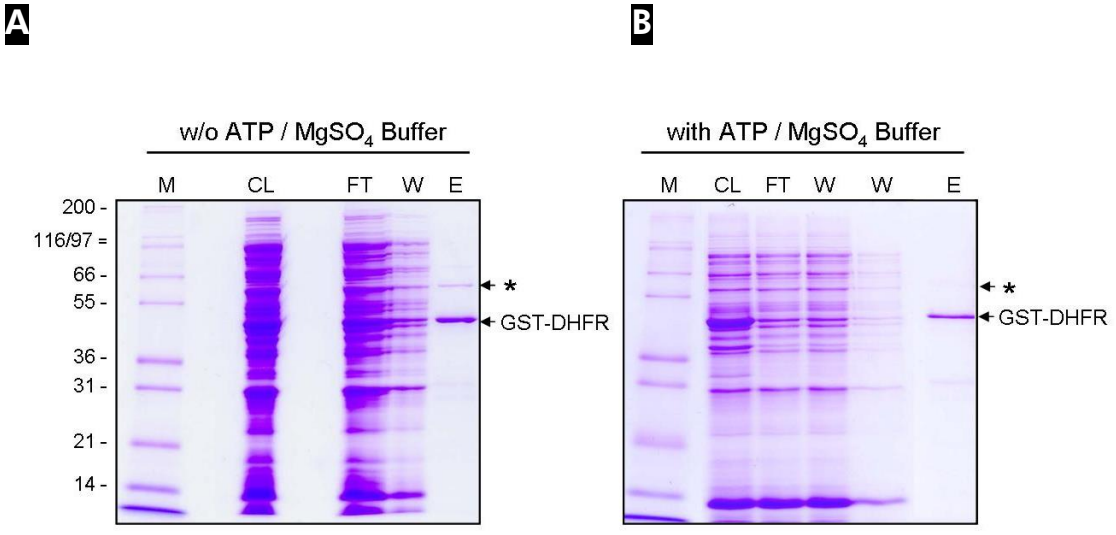


Figure 4. Removal of a prominent contaminant in glutathione affinity chromatography. *E. coli* cells overexpressing GST-DHFR were lysed in buffer PBS-L and binding to Glutathione HiCap Matrix was performed in lysis buffer. **A** Reactions were subjected to regular wash steps and **B** an additional ATP buffer wash step. Eluted protein was devoid of the 65–70 kDa contaminant (marked with an asterisk) when the buffer containing ATP and $MgSO_4$ was used. **CL**: Cleared lysate; **E**: Elution fraction; **FT**: Flow-through; **M**: Markers **W**: Wash.

Appendix C: Cleaning and Storage of Glutathione HiCap Matrix/Cartridges

Cleaning

Glutathione HiCap Cartridges and resin re-use depends upon the nature of samples applied and in general should only be performed with identical samples to prevent cross-contamination.

If Glutathione HiCap Matrix loses binding capacity or changes its color irreversibly, this may be due to accumulation of precipitated, denatured or nonspecifically bound components of the applied samples (proteins, lipids).

Denatured, precipitated or bound components may be removed as shown in Table 7.

Table 7. Cleaning agents for Glutathione HiCap Matrix

Component	Cleaning reagent
Precipitated, denatured and nonspecifically adsorbed components (mostly proteins)	6 M GuHCl or 0.1 M NaOH (2 bed volumes, low flow rate) 0.1M HCl
Hydrophobically bound components (e.g., lipids)	70% (v/v) ethanol or 1% (v/v) Triton X-100 (3–4 or 2 bed volumes, respectively, at low flow rate)

Immediately after cleaning, wash resin with 5 bed volumes of buffer PBS-EW. Resins can then be reused.

Storage

Wash resin or cartridge with 5 bed volumes of 20% (v/v) ethanol in water, close the column, and store at 2–8°C.

Appendix D: Optimization of Factor Xa-mediated Endoproteolytic Cleavage of GST from GST-Fusion Proteins

Protease concentration

Excess Factor Xa Protease may result in nonspecific proteolysis at secondary sites. Optimal enzyme specificity is achieved using the lowest amount of protease necessary to achieve complete cleavage.

Concentration of protein to be cleaved: Factor Xa Protease activity is sensitive to the concentration of protein to be cleaved. A minimum of 10 µg protein per 40 µl reaction (0.25 µg/µl) is recommended.

Incubation temperature

Factor Xa Protease activity increases with increasing incubation temperature from 4°C to 37°C. However, it should be considered that reduced incubation temperatures can minimize the accessibility of unwanted secondary cleavage sites.

pH

Factor Xa Protease activity decreases with increasing pH from pH 6.5 to pH 9.0. Therefore, we recommend a pH of 6.5 for the reaction buffer. If the individual protein is sensitive to pH, for example, with relation to protein activity or solubility, increase the pH to 7.5.

Buffer system

Use of phosphate buffers will result in reduced Factor Xa Protease activity compared with Tris·Cl or HEPES buffer systems.

Presence of detergents

Up to 1% Triton X-100, Nonidet P-40, and n-octylglucoside have no significant effect on Factor Xa Protease specificity or activity. The presence of SDS will significantly reduce enzyme activity.

Presence of denaturants

Significant loss of protease activity is observed at urea concentrations above 100 mM, and guanidine·HCl concentrations above 10 mM.

Presence of reductants

The presence of reductants in the reaction should be avoided, because the subunits of the heterodimeric protease enzyme are linked via a disulfide bridge. However, in some experiments up to 5 mM β-mercaptoethanol has been used with no significant influence on enzyme activity. The use of DTT is not recommended.

NaCl sensitivity

Factor Xa Protease activity decreases with increasing NaCl concentration. Significant inhibition is observed above 100 mM NaCl.

Anionic polymeric sugars

Some experiments have shown that addition of dextran sulfates (5000–10,000 Da) or heparin (5000 or 15,000 Da) to the reaction mixture results in increased Factor Xa Protease activity. 0.5–1 µg polymeric sugar can be added for cleavage of 10 µg recombinant protein in a 40 µl total reaction volume.

Amino acid sequence

Factor Xa Protease preferentially cleaves at the C-terminal side of the recognition sequence Ile-Glu-Gly-Arg.X. It has been reported that X is in general unspecific, but Ile and Thr are preferred. Where X is a hydrophobic residue, cleavage efficiency is reduced (15, 16).

References

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Cited references

(1) Simons, P.C., and Vander Jagt, D.L. (1977). Purification of glutathione S-transferase by glutathione affinity chromatography. Methods Enzymol. **77**, 235.

(2) Mannervik, B. (1985). The isoenzymes of glutathione S-transferase. Adv. Enzymol. **57**, 357.

(3) Smith, D.B., et al. (1988). Expression of an enzymatically active parasite molecule in *Escherichia coli*: *Schistosoma japonicum* glutathione S-transferase, Mol. Biochem. Parasitol. **27**, 249.

(4) Smith, D.B., and Johnson KS. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene. **67**, 31.

(5) McTigue, M.A., Williams, D.R., and Tainer, J.A. (1995). Crystal structure of a schistosomal drug and vaccine target: Glutathione S-transferase from *Schistosoma japonica* and its complex with the leading antischistosomal drug praziquantel. J. Mol. Biol. **246**, 21.

(6) Parker, M.W., Bello, M.L., and Federici, G. (1990). Crystallization of glutathione S-transferase from human placenta. J. Mol. Biol. **213**, 221.

(7) Ji, X., Zhang, P., Armstrong, R.N., and Gilliland, G.L. (1992). The three-dimensional structure of a glutathione S-transferase from the Mu gene class. Structural analysis of the binary complex of isoenzyme 3-3 and glutathione at 2.2-A resolution. Biochemistry. **231**,10169.

(8) Maru, Y., Afar, D.E., Witte, O.N., and Shibuya, M. (1996). The dimerization property of glutathione S-transferase partially reactivates Bcr-Abl lacking the oligomerization domain. J. Biol. Chem. **271**, 15353.

(9) Carter, P.S., and Homes, P. (2005). Automated purification of highly pure GST-tagged proteins using the BioRobot® Protein System — expression screening. QIAGEN, QIAGEN Newsletter 1. Available to download at www1.qiagen.com/literature/qiagennews/0105/1030329_QNews_0105_INT_lr.pdf#26.

(10) Dian, C., et al. (2002). Strategies for the purification and on-column cleavage of glutathione S-transferase fusion target proteins. J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. **769**, 133.

(11) Studier, F.W. (2005). Protein production by autoinduction in high-density shaking cultures. Prot. Expr. Purif. **41**, 207.

(12) *Ni-NTA Membrane Protein Kit Handbook*. QIAGEN, February 2009. Available for download at www.qiagen.com/protein.

(13) Einarson, M.B., and Orlinick, J.R. (2002). Identification of Protein-Protein Interactions with Glutathione S-Transferase Fusion Proteins. In: Protein-Protein Interactions: A Molecular Cloning Manual, Cold Spring Harbor Laboratory Press, p 37.

(14) Vikis, H.G., and Guan, K.-L. (2004). Glutathione-S-Transferase-Fusion Based Assays for Studying Protein-Protein Interactions. In Fu, H. ed., Protein-Protein Interactions, Methods and Applications, Methods in Molecular Biology. Humana Press, Totowa, N.J., p 175.

(15) Owen, W.G., Esmon, C.T., and Jackson, C.M. (1974). The conversion of prothrombin to thrombin. I. Characterization of the reaction products formed during the activation of bovine prothrombin. J. Biol. Chem. **249**, 594.

(16) He, M., Jin, L., and Austen, B. (1993). Specificity of factor Xa in the cleavage of fusion proteins. J. Protein Chem. **12**, 1.

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Glutathione HiCap Matrix (10 ml)	Glutathione HiCap Matrix (bed volume 10 ml)	30900
Glutathione HiCap Matrix (100 ml)	Glutathione HiCap Matrix (bed volume 100 ml)	30930
Glutathione HiCap Matrix (500 ml)	Glutathione HiCap Matrix (bed volume 5 x 100 ml)	30950
Glutathione HiCap Cartridge (5 x 1 ml)	5 cartridges pre-filled with 1 ml Glutathione HiCap Matrix: for automated purification of GST-tagged proteins using liquid chromatography systems	30971
Glutathione HiCap Cartridge (5 x 5 ml)	5 cartridges pre-filled with 5 ml Glutathione HiCap Matrix: for automated purification of GST-tagged proteins using liquid chromatography systems	30991
GST-tag Antibody (100 µg)	100 µg GST-tagged antibody, BSA-free, lyophilized	34860
Accessories		
Polypropylene Columns (1ml)	50/pack, 1 ml capacity	34924
Polypropylene Columns (5 ml)	50/pack, 5 ml capacity	34964
Factor Xa Protease	400 units Factor Xa Protease (2 units/µl)	33223
Xa Removal Resin	2 x 2.5 ml Xa Removal Resin, 3 x 1.9 ml 1 M Tris·Cl, pH 8.0	33213
Related products		
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