Glutathione **Sepharose** and prepacked columns

AFFINITY CHROMATOGRAPHY

Glutathione Sepharose™ 4B, Glutathione Sepharose 4 Fast Flow (FF), and Glutathione Sepharose High Performance (HP) are affinity chromatography media (resins) designed for purification of glutathione S-transferase (GST)-tagged proteins. The GST tag provides high binding specificity resulting in high purity in one step. There is also evidence that the GST tag can increase both solubility and stability of target proteins.

Purification of GST-tagged proteins can be performed under very mild conditions, which preserves protein function and antigenicity. GST-tagged proteins that are expressed using, for example, pGEX vectors, can be purified directly from pretreated bacterial lysates.

GST is a large protein tag (M_r 26 000) and forms a dimer under normal purification conditions and therefore, tag cleavage is often needed. The binding kinetics between GST and glutathione is slow and therefore a low flow rate during sample application is recommended.

Key benefits of Glutahione Sepharose and prepacked columns are:

- Fast, one-step purification of GST-tagged proteins
- Prepacked GSTrap™ and GSTPrep™ columns for convenience and high reproducibility

Chromatography medium characteristics

The glutathione ligand is coupled to Sepharose via a 10-carbon linker. The three media differ with respect to cross-linking and bead size to suit different needs:

- Glutathione Sepharose 4B provides the highest binding capacity and recovery
- Glutathione Sepharose 4 FF has improved flow properties for faster purifications and scale-up
- Glutathione Sepharose HP provides high resolution, resulting in high concentration of eluted target protein even at low load

Fig 1. Glutathione Sepharose affinity chromatography media are available in a variety of different pack sizes as well as in prepacked GSTrap (1 and 5 ml) and GSTPrep FF 16/10 columns.

The smaller bead size of Glutathione Sepharose HP, 34 µm, results in narrower and sharper peaks. A description of the characteristics for each medium is given in Table 1.

Table 1. Characteristics of Glutathione Sepharose media

	Glutathione Sepharose 4B	Glutathione Sepharose 4 FF	Glutathione Sepharose HP
Matrix	4% agarose	Highly cross-linked 4% agarose	Highly cross-linked 6% agarose
Ligand	Glutathione	Glutathione	Glutathione
Mean particle size	90 µm	90 µm	34 µm
Binding capacity*	> 25 mg/ml medium	> 10 mg/ml medium	> 7 mg/ml medium
Chemical stability	All commonly used aqueous buffers, e.g., 1 M acetate, pH 4.0 and 6 M guanidine-HCl for 1 h at room temperature	All commonly used aqueous buffers, e.g., 1 M acetate, pH 4.0 and 6 M guanidine-HCl for 1 h at room temperature	All commonly used aqueous buffers, e.g., 1 M acetate, pH 4.0 and 6 M guanidine-HCl for 1 h at room temperature
Recommended flow rate	Sample loading <100 cm/h; wash and elution 100 to 300 cm/h	Sample loading <100 cm/h; wash and elution 100 to 300 cm/h	Sample loading <100 cm/h; wash and elution 100 to 300 cm/h
pH stability	4 to 13	3 to 12	3 to 12
Storage	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol	4°C to 30°C in 20% ethanol

* Binding of GST to glutathione is protein-to-protein and flow-rate dependent. Lower flow rates often increase the binding capacity. This is important to consider during sample application.

Prepacked columns characteristics

All Glutathione Sepharose media are available in convenient, prepacked HiTrap™ formats (GSTrap), and Glutathione Sepharose FF is also available in HiPrep™ format (GSTPrep) for facilitated scale-up (Table 2). GSTrap and GSTPrep prepacked columns can be used with ÄKTA™ chromatography systems and UNICORN™ software which include preset method templates for these columns. The combination of prepacked GSTrap and GSTPrep prepacked columns with ÄKTA chromatography systems ensures that higher reproducibility is achieved in shorter time.

The prepacked columns are made of transparent, biocompatible polypropylene that does not interact with biomolecules. GSTrap 4B/FF/HP and GSTPrep FF 16/10 columns are not designed to be opened or repacked.

Operation

GSTrap and GSTPrep columns are quick and easy to use. All columns are designed for use with laboratory pumps or an ÄKTA chromatography system. Manual purification can also be easily performed with GSTrap columns using a syringe and the provided Luer connector. Instructions are included in all packages.

Scale-up

To increase the binding capacity, two or more GSTrap 1 ml or 5 ml columns can be connected in series by screwing the end of one into the top of the next column. Note that connection of columns in series will cause an increase in back pressure. This is easily addressed by lowering the flow rate. GSTPrep FF 16/10 (20 ml) is packed with Glutathione Sepharose 4 FF, which has improved flow and stability properties and is recommended for larger scale purifications. When further scale-up is necessary, bulk quantities are available for packing of larger columns.

Table 2. Characteristics of GSTrap 4B/FF/HP and GSTPrep FF 16/10 columns

	GSTrap 4B/FF/HP (1 ml)	GSTrap 4B/FF/HP (5 ml)	GSTPrep FF 16/10
Bed volume	1 ml	5 ml	20 ml
Bed height	2.5 cm	2.5 cm	10 cm
Column i.d.	0.7 cm	1.6 cm	1.6 cm
Column hardware	Polypropylene	Polypropylene	Polypropylene
Recommended flow rate:			
Sample loading	0.3 to 0.5 ml/min	1.5 to 2.5 ml/min	2 to 3 ml/min
Wash and elution	4B and HP: 1 ml/min FF: 1.5 ml/min	4B and HP: 5 ml/min FF: 7 ml/min	8 ml/min
Maximum flow rate	4 ml/min	15 ml/min	10 ml/min (300 cm/h)
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)	5 bar (0.5 MPa, 70 psi)	5 bar (0.5 MPa, 70 psi)

Application 1: Comparison of different Glutathione Sepharose media

The three different Glutathione Sepharose media have different characteristics, which are illustrated in this experiment. Clarified *E. coli* lysate (20 ml) expressing GST-hippocalcin was loaded to GSTrap 4B, GSTrap HP, and GSTrap FF (Fig 2). The yield was calculated from absorbance at 280 nm, and the purity analyzed with SDS-PAGE using Deep Purple™ Total Protein Stain imaged with Typhoon™ variable mode imager (Fig 3). The results clearly demonstrate the high capacity of GSTrap 4B and the higher resolution of GSTrap HP. Glutathione Sepharose 4 FF, which is the medium prepacked in GSTrap FF columns, is the best choice for scale-up (see *Application 3*).

Sample:	20 ml clarified *E. coli* lysate expressing GST-hippocalcin
Flow rate:	Sample application: 0.3 ml/min Equilibration, wash, and elution: 1 ml/min
Binding buffer:	PBS, pH 7.4
Elution buffer:	50 mM Tris-HCl, 10 mM glutathione, pH 8

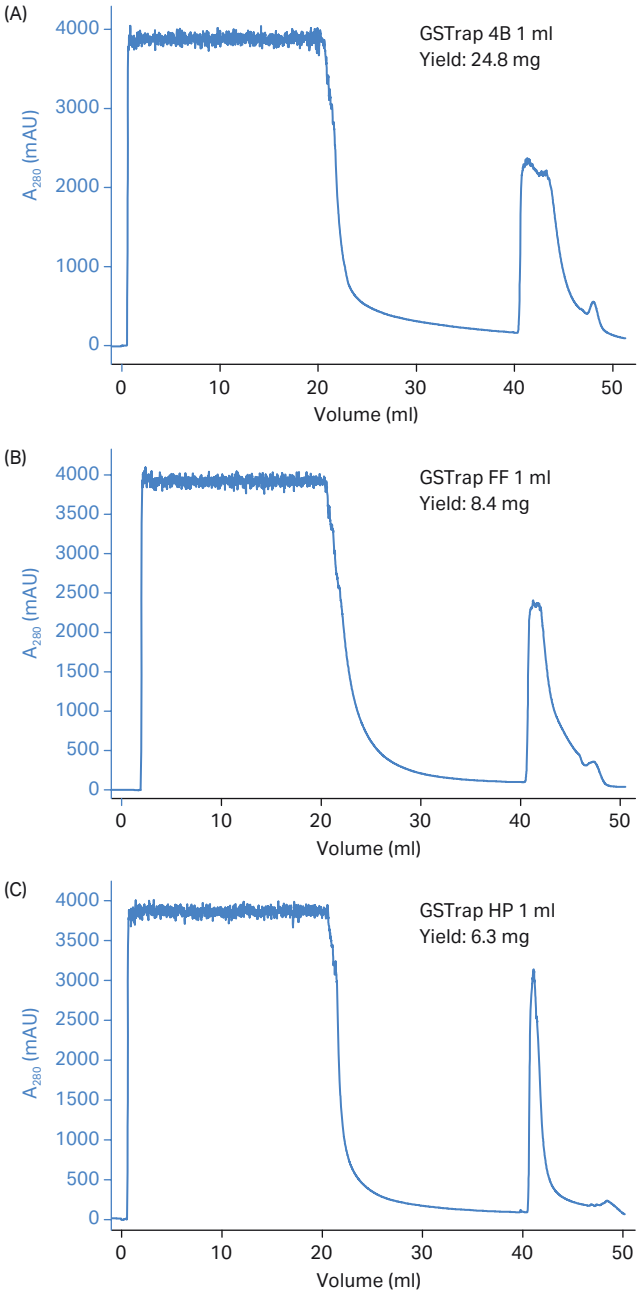


Fig 2. Comparison of purifications of GST-hippocalcin on (A) GSTrap 4B, (B) GSTrap FF, and (C) GSTrap HP.

Lanes

1 LMW markers (LMW-SDS Marker Kit)
2 Starting material
3 Eluate from GSTrap 4B
4 Flowthrough from GSTrap 4B
5 Wash GSTrap 4B
6 Eluate from GSTrap HP
7 Flowthrough from GSTrap HP
8 Wash GSTrap HP
9 Eluate from GSTrap FF
10 Flowthrough from GSTrap FF
11 Wash GSTrap FF

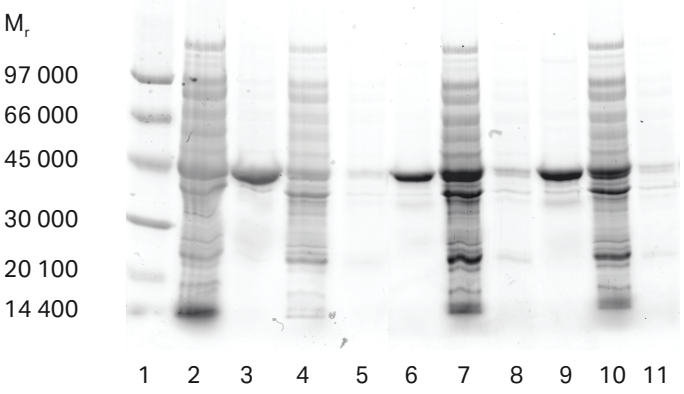


Fig 3. SDS-PAGE analysis of GST-hippocalcin purification. Electrophoresis was performed using ExcelGel™ SDS Gradient 8-18 on Multiphor™ II. Staining was performed using Deep Purple Total Protein Stain and image analysis performed using Typhoon.

Application 2: Yield of GST-tagged protein with Glutathione Sepharose 4B

The expected yield from purification of GST-tagged proteins is dependent on both protein and sample properties, as well as purification conditions. Yields are, however, generally high with Glutathione Sepharose 4B. Three different proteins were purified on GSTrap 4B 1 ml using varied sample concentrations, sample volumes, and total loads (Table 3). To obtain desired concentration, the proteins were purified and then spiked into untransformed *E. coli* lysates to the desired concentrations. Flow rate during sample application was 0.3 ml/min, and during wash and elution 1 ml/min. The binding buffer was PBS, pH 7.4.

Yields were calculated from absorbance at 280 nm (Table 3 and Fig 4), and the purity analyzed with SDS-PAGE (data not shown). The recovery of the different GST-tagged proteins vary between proteins and sample conditions but is generally >60%.

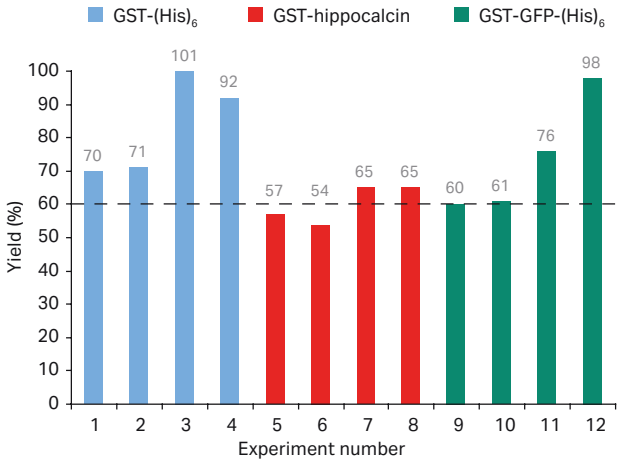


Fig 4. Yield (%) of three different GST-tagged proteins during purifications on Glutathione Sepharose 4B. Yields are generally over 60% (dashed line), see Table 3 for more details.

Table 3. Experimental conditions for determination of yields in protein purification using Glutathione Sepharose 4B

	GST-$(His)_6$ (M_r 26 000)				GST-hippocalcin (M_r 45 000)				GST-GFP-$(His)_6$ (M_r 55 000)			
Experiment	1	2	3	4	5	6	7	8	9	10	11	12
Conc. of GST-tagged protein (mg/ml)	0.05	0.1	0.5	0.5	0.05	0.12	0.5	0.6	0.05	0.1	0.5	0.5
Sample volume (ml)	10	60	1	12	10	100	1	20	10	100	1	20
Total load (mg)	1	6	1	6	1	12	1	12	0.5	10	0.5	10
Yield (%)	70	71	101	92	57	54	65	65	60	61	76	98

Application 3: Purification and scale-up of GST-DemA using Glutathione Sepharose 4 FF

Glutathione Sepharose 4 FF is the optimal media for scale-up. The purification of GST-DemA was scaled-up from GSTrap FF 1 ml to GSTrap FF 5 ml and GSTPrep FF 16/10. The gene encoding DemA was isolated from *Streptococcus dysgalactiae.* DemA is a fibrinogen-binding protein that shows both plasma protein binding properties and sequence similarities with the M and M-like proteins of other *Streptococcus* species.

Preparation of cytoplasmic extract

E. coli expressing GST-tagged DemA was resuspended in PBS, pH 7.2 (1 g/5 ml) supplemented with 1 mM PMSF, 1 mM DTT, 100 mM $MgCl_2$, 1 U/ml RNase A, and 13 U/ml DNase I. The cells were sonicated on ice with a Vibra-Cell™ ultrasonic processor for 3 min at 50% amplitude. Cell debris was removed by centrifugation at 48 000 × g at 4°C for 30 min. The supernatant was loaded to the column after passage through a 0.45 µm filter.

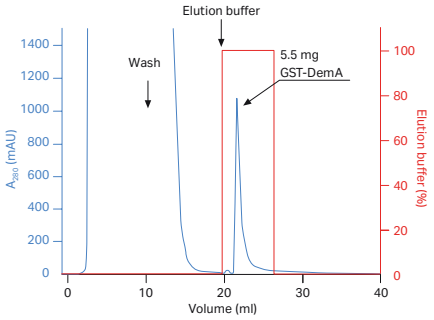
Purification and scale-up

The purification of GST-DemA was performed using an ÄKTA chromatography system. The columns were equilibrated before sample was loaded on GSTrap FF 1 ml (Fig 5A), GSTrap FF 5 ml (Fig 5B), and GSTPrep FF 16/10 (Fig 5C). After wash, the proteins were eluted and analyzed by SDS-PAGE (Fig 6).

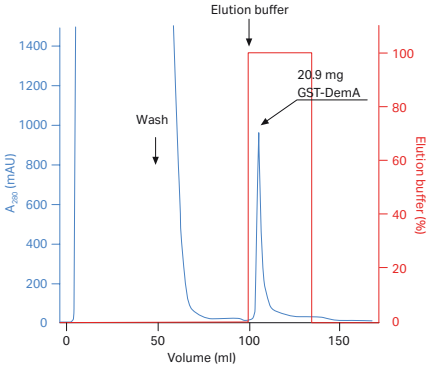
The most important parameter in this scale-up study is the residence time; that is the period of time the sample is in contact with the chromatography medium. The residence time was the same for the GSTrap FF 1 ml and 5 ml columns, while it was twice as long for the GSTPrep FF 16/10 column due to the difference in column length versus column diameter.

Some of the applied protein was found in the flowthrough, an effect of the low adsorption rate of GST. The amount of eluted GST-tagged proteins increased proportionally with increased column volume and sample load.

(A) *Column:* GSTrap FF 1 ml
Sample: 10 ml extract from *E. coli* expressing GST-DemA
Binding buffer: PBS, pH 7.4
Elution buffer: 50 mM Tris-HCl, pH 8.0, with 10 mM reduced glutathione
Flow rate: Sample loading: 0.5 ml/min
Washing and elution: 1 ml/min
Procedure: 5 CV (CV = column volumes) binding buffer, 10 ml sample loaded, 10 CV binding buffer, 7 CV elution buffer, and 5 CV binding buffer



(B) *Column:* GSTrap FF 5 ml
Sample: 50 ml extract from *E. coli* expressing GST-DemA
Binding buffer: PBS, pH 7.4
Elution buffer: 50 mM Tris-HCl, pH 8.0, with 10 mM reduced glutathione
Flow rate: Sample loading: 2.5 ml/min
Washing and elution: 5 ml/min
Procedure: 5 CV binding buffer, 50 ml sample loaded, 10 CV binding buffer, 7 CV elution buffer, and 5 CV binding buffer



(C) *Column:* GSTPrep FF 16/10
Sample: 200 ml extract from *E. coli* expressing GST-DemA
Binding buffer: PBS, pH 7.4
Elution buffer: 50 mM Tris-HCl, pH 8.0, with 10 mM reduced glutathione
Flow rate: Sample loading: 5 ml/min
Washing and elution: 10 ml/min
Procedure: 5 CV binding buffer, 200 ml sample, 10 CV binding buffer, 7 CV elution buffer, and 5 CV binding buffer

Note: Data was obtained using first-generation GSTPrep FF 16/10 columns.

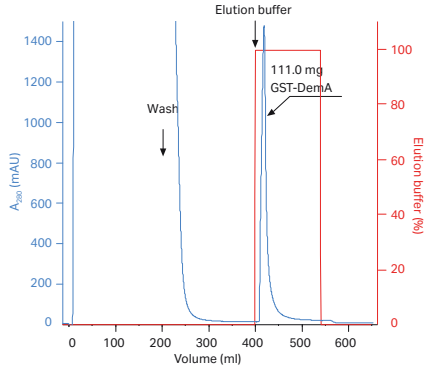


Fig 5. Purification and scale-up of GST-DemA on (A) GSTrap FF 1 ml, (B) GSTrap FF 5 ml, and (C) GSTPrep FF 16/10.

Lanes

1 LMW markers, reduced (LMW-SDS Marker Kit)
2 Extract of *E. coli* expressing GST-DemA, 1 g cell paste/5 ml
3 Flowthrough from GSTrap FF 1 ml
4 GST-DemA eluted from GSTrap FF 1 ml
5 Extract of *E. coli* expressing GST-DemA, 1 g cell paste/5 ml
6 Flowthrough from GSTrap FF 5 ml
7 GST-DemA eluted from GSTrap FF 5 ml
8 Extract of *E. coli* expressing GST-DemA, 1 g cell paste/5 ml
9 Flowthrough from GSTPrep FF 16/10
10 GST-DemA eluted from GSTPrep FF 16/10

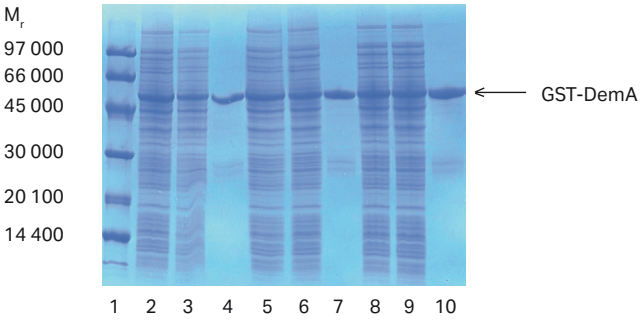


Fig 6. SDS-PAGE analysis of GST-DemA on ExcelGel Homogeneous 12.5% using Multiphor II followed by Coomassie™ staining. Due to the low turnover rate of GST, some of the applied protein was found in the flowthrough.

Application 4: Automated multistep purification and tag removal

Removal of the GST tag from the target protein is often necessary due to its large size. GST-tagged proteins produced using one of the pGEX expression vectors can be purified and the tag cleaved directly on-column in one step using a protease such as PreScission™ Protease. The advantage of on-column tag removal is that it eliminates the extra step of separating the cleaved protein from GST since the GST tag itself remains bound to the medium. PreScission Protease is also GST-tagged, and during elution, the protease will remain bound to the medium, providing high purity of the eluted target protein. PreScission Protease has the added benefit of improving target protein stability since it is maximally active at 4°C.

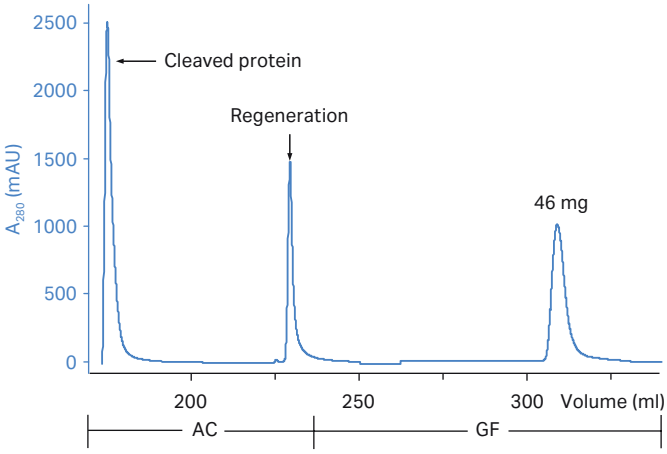
Automated tag cleavage on ÄKTAxpress

E. coli lysate (32 ml) containing approximately 50 mg of GST-pur-α was loaded onto a GSTrap HP 5 ml column connected to ÄKTAxpress. Wash was performed with binding/cleavage buffer and 20 U PreScission Protease/mg GST-tagged protein diluted in binding buffer was added. Cleavage was performed on-column for 8 h at 4°C. After elution of the cleaved protein, the affinity column was regenerated and the GST tag, PreScission Protease, and remaining uncleaved protein were collected in a separate outlet and discarded.

GST-pur-α was successfully purified and the tag cleaved off using automated multistep purification on ÄKTAxpress. Both yield and purity were satisfactory.

(A)

Sample:	*E. coli* lysate (32 ml) containing GST-Pur-α (M_r 61 600)
Affinity chromatography:	GSTrap HP 5 ml
Binding/cleavage buffer:	50 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, pH 7.5
Elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione pH 8.0
Gel filtration:	HiLoad™ 16/60 Superdex™ 75 pg, 120 ml
Buffer:	50 mM Tris-HCl, 150 mM NaCl, pH 7.5
System:	ÄKTAxpress
Running conditions:	4°C
Incubation time:	8 h
Amount protease loaded:	20 U PreScission Protease/mg GST-Pur-α



(B) **Lanes**

1 LMW markers, LMW-SDS Marker Kit
2 Start material
3 Flowthrough
4 Purified, cleaved pur-α
5 Uncleaved GST-pur-α

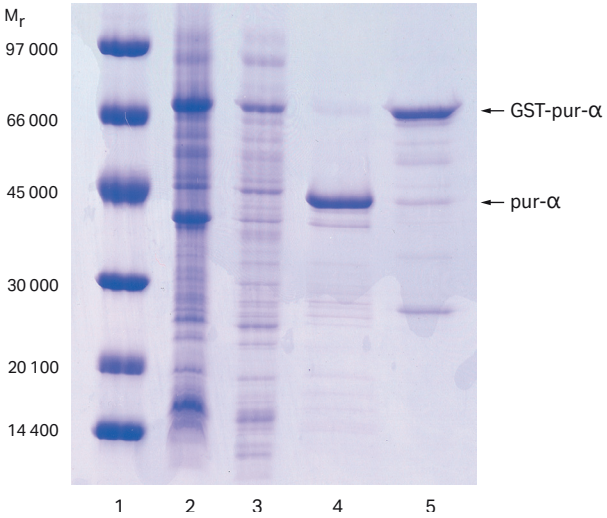


Fig 7. (A) Two-step protocol for automatic GST-tagged protein cleavage with PreScission Protease and purification on ÄKTAxpress. (B) Analysis of the untagged target protein after purification and GST-tagged cleavage on SDS-PAGE and Coomassie staining.

Ordering information

Product	Quantity	Code number
Glutathione Sepharose 4B	10 ml	17-0756-01
	100 ml	17-0756-05
	300 ml	17-0756-04
Glutathione Sepharose 4 Fast Flow	25 ml	17-5132-01
	100 ml	17-5132-02
	500 ml	17-5132-03
Glutathione Sepharose High Performance	25 ml	17-5279-01
	100 ml	17-5279-02
GSTPrep FF 16/10	1 × 20 ml	28-9365-50
GSTrap 4B	1 × 1 ml	29-0486-09
	5 × 1 ml	28-4017-45
	1 × 5 ml	28-4017-47
	5 × 5 ml	28-4017-48
GSTrap FF	2 × 1 ml	17-5130-02
	5 × 1 ml	17-5130-01
	1 × 5 ml	17-5131-01
	5 × 5 ml	17-5131-02
GSTrap HP	5 × 1 ml	17-5281-01
	1 × 5 ml	17-5282-01
	5 × 5 ml	17-5282-02

Related products	Quantity	Code number
GST Detection Module	50 reactions	27-4590-01
Glutathione S-transferase gene fusion vectors (pGEX vectors)*	Various	Various
GST MultiTrap™ FF	4 × 96-well plates	28-4055-01
GST MultiTrap 4B	4 × 96-well plates	28-4055-00
Anti-GST Antibody	0.5 ml	27-4577-01
GST SpinTrap™	50 columns	28-9523-59
GST GraviTrap™	10 columns	28-9523-60

* Visit www.cytiva.com/pgex for more information

Site-specific proteases	Quantity	Code number
PreScission Protease	500 units	27-0843-01
Thrombin	500 units	27-0846-01
Factor Xa	400 units	27-0849-01

Empty lab-scale columns	Quantity	Code number
Tricorn™ 5/20 column	1	28-4064-08
Tricorn 5/50 column	1	28-4064-09
Tricorn 10/20 column	1	28-4064-13
Tricorn 10/50 column	1	28-4064-14
Tricorn 10/100 column	1	28-4064-15
XK 16/20 column	1	18-8773-01
XK 16/40 column	1	18-8774-01
XK 26/20 column	1	18-1000-72
XK 26/40 column	1	18-8768-01

Accessories	Quantity	Code number
1/16″ male/Luer female*	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16″ female/M6 male	6	18-1112-57
Union M6 female/1/16″ male	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16″ male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16″†	5	11-0004-64
Fingertight stop plug, 1/16″‡	5	11-0003-55

* One connector included in each HiTrap package.
† Two, five, or seven stop plugs female included in HiTrap packages depending on products.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature	Code number
GST Gene Fusion System, Handbook	18-1157-58
Recombinant Protein Purification, Principles and Methods Handbook	18-1142-75
Affinity Chromatography, Principles and Methods Handbook	18-1022-29
Solutions for protein preparation and detection of GST-tagged proteins, Selection guide	28-9168-33
Prepacked chromatography columns for ÄKTA systems, Selection guide	28-9317-78

cytiva.com/protein-purification

For local office contact information, visit cytiva.com/contact

CY13687-10Aug20-DF