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Affinity chromatography

Glutathione Sepharose High Performance GSTrap HP

Glutathione Sepharose™ High Performance is a high performance affinity medium for the purification of GST-tagged proteins.

Its performance characteristics include:

- Glutathione coupled to Sepharose High Performance, excellent for high resolution purification of GST-tagged proteins
- Easy one-step purification of GST-tagged proteins and other glutathione S-transferase or glutathione-dependent proteins
- Prepacked GSTrap™ HP columns for simple operation with a syringe, pump or chromatographic system, such as ÄKTAdesign™

Fig 1. Glutathione Sepharose High Performance and prepacked GSTrap HP 1-ml and 5-ml columns.

These features make Glutathione Sepharose High Performance the ideal medium for high resolution purification of GST-tagged proteins, glutathione S-transferases and glutathione binding proteins. The prepacked HiTrap™ columns are simple and convenient to use, whereas the laboratory packs give you the flexibility of choosing column type and size.

GST-tagged proteins are purified from clarified bacterial lysates using immobilized glutathione, and are eluted under mild, non-denaturing conditions to preserve the function of the target protein. The GST-tag, binds to the ligand glutathione coupled on Sepharose High Performance. GST-tagged proteins can be detected using colorimetric or immunological methods.

The GST-tag is often used to increase the yield of expression and solubility of the target protein, however, removal of the GST-tag from the target protein is often necessary due to its large size. A GST-tagged protein produced using one of the pGEX expression vectors can be purified directly on-column in one step by cleaving the tag with 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. The PreScission Protease is GST-tagged and during elution the protease will remain bound to the medium providing high purity of the target protein. PreScission Protease has the added benefit of improving target protein stability since it is maximally active at 4°C. Moreover; the specific cleavage sequence prevents cleavage of the target protein.

Medium characteristics

The glutathione ligand is coupled via a 10-carbon linker to highly cross-linked 6% agarose. The coupling is optimized to give a high binding capacity for GST-tagged proteins and other glutathione binding proteins. The medium, Glutathione Sepharose High Performance, has a small bead size (34 µm).

Figure 2 shows overlaid chromatograms to compare Glutathione Sepharose 4 Fast Flow (bead size 90 µm) with Glutathione Sepharose High Performance in a purification of GST-His (M_r 26 000), expressed in *E. coli*. The Glutathione Sepharose High Performance peaks are both narrower and sharper which indicates a more concentrated sample.

The dynamic binding capacity is > 10 mg GST-tagged protein/ml medium. The dynamic binding capacity will vary depending on several factors such as target protein, flow rate, pH, temperature, etc. Table 1 lists the main characteristics of Glutathione Sepharose High Performance.

Columns:	GSTrap HP 1 ml and GSTrap FF 1 ml
Sample:	GST-His, M_r 26 000
Sample pretreatment:	*E. coli* cells were lysed by sonication and clarified by centrifugation, 18 000 rpm 20 min (approx. 40 000 x g)
Binding buffer:	50 mM Tris-HCl, 0.15 M NaCl, pH 7.5
Elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Flow rate	
sample loading:	0.5 ml/min
wash and elution:	2 ml/min
Running temperature:	Room temperature

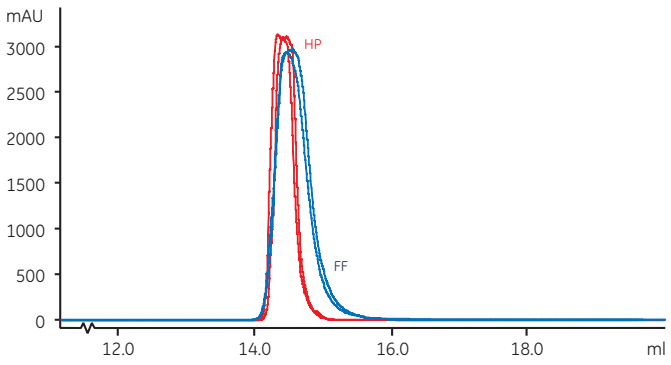


Fig 2. Comparison of Glutathione Sepharose High Performance with Glutathione Sepharose 4 Fast Flow.

Note: Only the eluted peaks are shown.

Table 1. Main characteristics of Glutathione Sepharose High Performance

Matrix	Highly cross-linked 6% agarose
Average particle size	34 µm
Ligand	Glutathione
Dynamic binding capacity[1]	> 10 mg GST-tagged protein/ml medium, M_r 63 000
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
Recommended flow rate[1]	Sample loading: 30–150 cm/h (1–5 ml/min using XK 16/20 column) Wash and elution: 150–300 cm/h (5–10 ml/min using XK 16/20 column)
Maximum back pressure	0.3 MPa (3 bar, 43 psi)
Maximum flow rate[2]	600 cm/h
pH stability	pH 3–12
Storage temperature	4°C to 30°C
Storage	20% ethanol

[1] Dynamic binding capacity conditions ($QB_{60\%}$) at room temp.

Sample:	1 mg/ml pure GST-tagged protein in binding buffer
Column volume:	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

Note: Binding of GST to glutathione is flow rate dependent and lower flow rates often increase the binding capacity. The binding capacity is also dependent on the target protein.

[2] H_2O at room temperature.

GSTrap HP - prepacked Glutathione Sepharose High Performance columns

Glutathione Sepharose High Performance is available in prepacked GSTrap HP 1-ml and 5-ml columns (Fig 1) to save time and to give high reproducibility.

GSTrap HP columns are made of transparent, polypropylene that does not interact with biomolecules. They have porous top and bottom frits that allow high flow rates. The columns are delivered with a set of connectors to allow simple connection to different chromatography systems. Moreover, when GSTrap HP columns are used in combination with ÄKTAdesign systems, preprogrammed method templates and wizards based on these columns give you a fast and easy start to your purification.

Table 2 lists the main characteristics of GSTrap HP columns.

GSTrap HP columns are not designed to be opened or to be repacked.

Scale-up

Two or more prepacked GSTrap HP 1-ml or 5-ml columns can easily be connected in series by screwing the end of one into the top of the next to increase the binding capacity (back-pressure will increase). Glutathione Sepharose High Performance is also available in 25 ml or 100 ml lab pack sizes for packing larger columns such as Tricorn™ or XK glass columns (see Ordering Information).

Table 2. Characteristics of GSTrap 1-ml and 5-ml columns

Column dimensions (i.d. × h)	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Column volumes	1 ml and 5 ml
Medium	Glutathione Sepharose High Performance
Dynamic binding capacity[1]	> 10 mg GST-tagged protein/ml medium, M_r 63 000
Chemical stability	All commonly used aqueous buffers, e.g. 1 M acetate, pH 4.0 and 6 M guanidine hydrochloride for 1 h at room temperature
Recommended flow rates[1]	
Sample loading:	0.2–1 ml/min (1 ml) and 1–5 ml (5 ml)
Wash and elution:	1–2 ml/min (1 ml) and 5–10 ml/min (5 ml)
Maximum flow rate	4 ml/min and 15 ml/min for 1-ml and 5-ml columns respectively
Maximum back pressure	0.3 MPa, 3 bar
pH stability	pH 3–12
Storage temperature	4°C to 30°C
Storage	20% ethanol

[1] Dynamic binding capacity conditions ($QB_{10\%}$) at room temp.
Sample: 1 mg/ml pure GST-tagged protein in binding buffer
Column volume: 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
Note: Binding of GST to glutathione is flow rate dependent and lower flow rates often increase the binding capacity. The binding capacity is also dependent on the target protein.

Operation

GSTrap HP columns are designed for fast and easy use with pumps or chromatography systems, such as ÄKTAdesign.

Manual purification with GSTrap HP columns is also easily performed with a syringe (connectors are provided). Figure 3 illustrates this technique. Instructions are included in all packages.

Applications

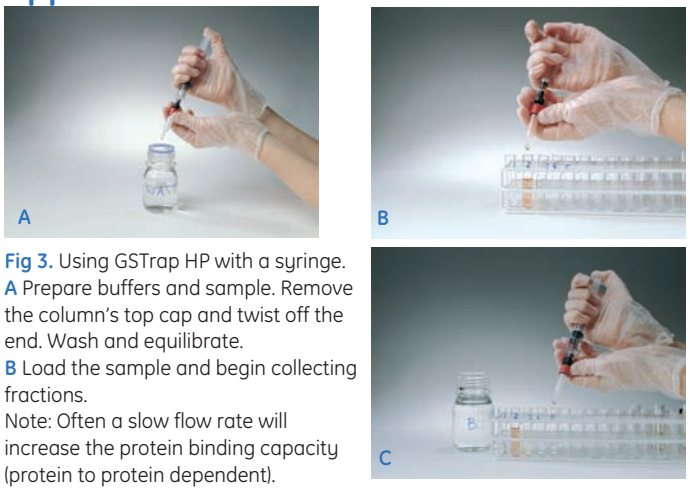


Fig 3. Using GSTrap HP with a syringe.
A Prepare buffers and sample. Remove the column's top cap and twist off the end. Wash and equilibrate.
B Load the sample and begin collecting fractions.
Note: Often a slow flow rate will increase the protein binding capacity (protein to protein dependent).
C Elute and continue to collect fractions.

Glutathione Sepharose High Performance is easy to use for one-step purification of GST-tagged proteins. The following applications show data from purification, reproducibility, purity and scale-up experiments with GSTrap HP 1 ml and 5 ml.

Single step purification of GST-hippocalcin

This application shows a one-step purification of GST-hippocalcin (M_r 43 000), expressed in *E. coli*, and purified with a GSTrap HP 1-ml column (Fig 4a and Fig 4b). The purity was determined by SDS-PAGE.

Stability and reproducibility

Column: GSTrap HP 1 ml
Sample: Clarified *E. coli* homogenate containing expressed GST-hippocalcin, M_r 43 000
Binding buffer: 10 mM sodium phosphate, 0.14 M NaCl, 1 mM DTT, pH 7.5
Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, 1 mM DTT, pH 8.0
Flow rate
sample loading: 0.3 ml/min
wash and elution: 1 ml/min
Running temperature: Room temperature

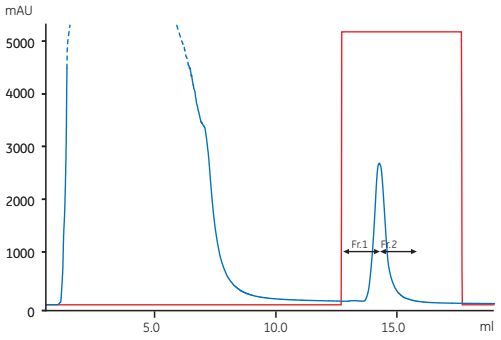


Fig 4a. One-step purification of GST-hippocalcin on GSTrap HP 1 ml

Lane 1: Low Molecular Markers
Lane 2: Clarified *E. coli* homogenate containing expressed GST-hippocalcin
Lane 3: Flow through, diluted 1:40
Lane 4: Washed out unbound sample, diluted 1:40
Lane 5: Eluted fraction 1, diluted 1:8
Lane 6: Eluted fraction 2, diluted 1:8
Lane 7: Pool of eluted fractions 1 and 2, diluted 1:12

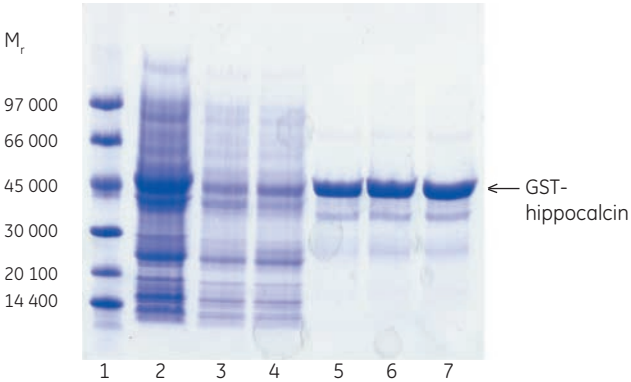


Fig 4b. Coomassie™ stained non-reduced SDS-PAGE (ExcelGel™ SDS Gradient 8–18) of fractions from purification shown in Fig 4a.

To check the stability of the medium and the reproducibility of purification, *E. coli* homogenates containing GST-hippocalcin were repeatedly purified ten times in the same column without cleaning between runs.

The ten overlaid chromatograms show a near perfect match indicating little or no variation in binding capacity and stability of the medium. SDS-PAGE analysis also indicates no changes in purity or binding after ten runs (Figs 5a and 5b).

Column: GSTrap HP 1 ml
Sample: Clarified *E. coli* homogenate containing expressed GST-hippocalcin, M_r 43 000
Binding buffer: 10 mM sodium phosphate, 0.14 M NaCl, 1 mM DTT, pH 7.5
Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, 1 mM DTT, pH 8.0
Flow rate
sample loading: 0.3 ml/min
wash and elution: 1 ml/min
Running temperature: Room temperature

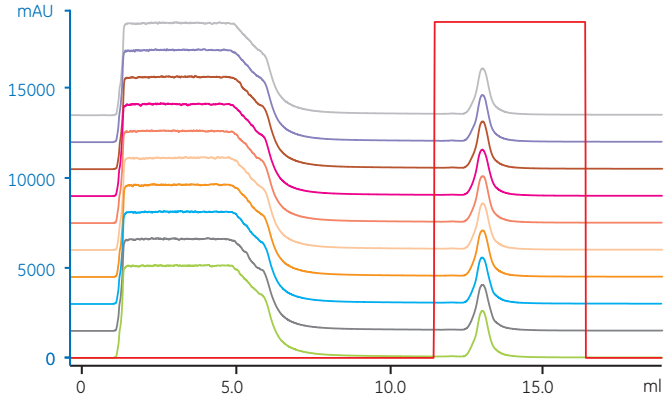
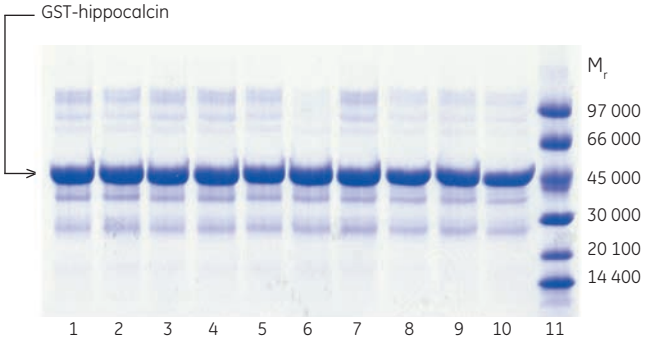


Fig 5a. Confirmation of the stability of Glutathione Sepharose High Performance. Chromatographic overlay of ten repetitive purifications.



Lanes 1-10: Eluted pooled fractions from runs 1– 10, diluted 1:8
Lane 11: Low Molecular Markers

Fig 5b. Coomassie stained non-reduced SDS-PAGE (ExcelGel SDS Gradient 8–18) of pooled fractions from repetitive purification runs shown in Figure 5a.

Scale-up

In a scale-up study, 5 ml and 25 ml of *E. coli* homogenate containing GST-hippocalcin was loaded on GSTrap HP 1-ml and 5-ml columns respectively. Figure 6 shows the chromatograms from the two runs. The amount of protein in the eluted peaks was calculated to 6.5 mg and 39.7 mg respectively. The results show the simplicity of scale-up with prepacked columns. Analysis is done with SDS-PAGE (Fig 7).

Columns: GSTrap HP 1 ml and GSTrap HP 5 ml
Sample: Clarified *E. coli* homogenate containing expressed GST-hippocalcin, M_r 43 000
Sample volumes: GSTrap HP 1 ml: 5 ml
5 ml: 25 ml
Binding buffer: 10 mM sodium phosphate, 0.14 M NaCl, pH 7.4
Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Flow rate
sample loading: GSTrap HP 1 ml: 0.3 ml/min
5 ml: 1.6 ml/min
wash and elution: GSTrap HP 1 ml: 1 ml/min
5 ml: 4 ml/min
Running temperature: Room temperature

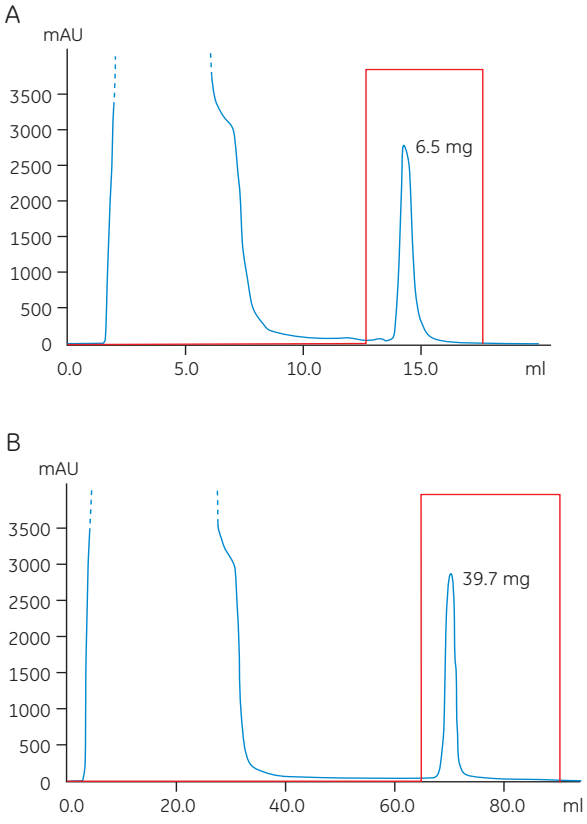
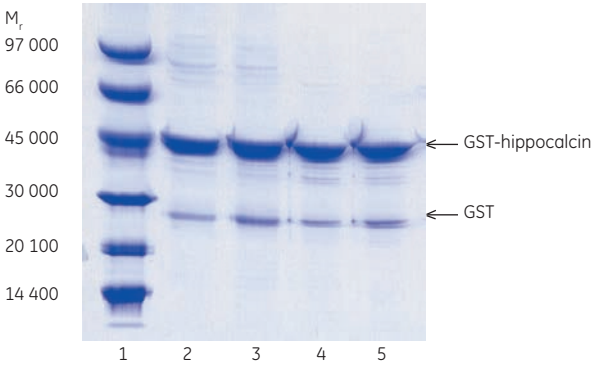


Fig 6 Scale-up from GSTrap HP 1 ml (A) to GSTrap HP 5 ml (B)

The SDS-PAGE shows GST-hippocalcin analyzed under non-reducing and reducing buffer conditions (Fig 7). Each well was loaded with 10 µg protein. The SDS-PAGE also shows that free GST is expressed.



Lane 1: Low Molecular Markers
Lane 2: Eluted pool from GSTrap HP 1 ml, non-reduced
Lane 3: " 5 ml, non-reduced
Lane 4: " 1 ml, reduced
Lane 5: " 5 ml, reduced

Fig 7. Coomassie stained reduced and non-reduced SDS-PAGE (ExcelGel SDS Gradient 8-18) of fractions from purification shown in Figure 6.

Storage

Glutathione Sepharose High Performance and GSTrap HP 1 ml and 5 ml are supplied in 20% ethanol. The recommended storage temperature is 4°C to 30°C.

Further information

Visit *www.gehealthcare.com/hitrap* for more information regarding the GSTrap HP and other HiTrap columns. For more information about all laboratory purification media, visit *www.gehealthcare.com/protein-purification* or contact your local representative.

Ordering information

Product	Quantity	Code No.
GSTrap HP	5 × 1 ml	17-5281-01
GSTrap HP	100 × 1 ml*	17-5281-05
GSTrap HP	1 × 5 ml	17-5282-01
GSTrap HP	5 × 5 ml	17-5282-02
GSTrap HP	100 × 5 ml*	17-5282-05
Glutathione Sepharose High Performance	25 ml	17-5279-01
Glutathione Sepharose High Performance	100 ml†	17-5279-02

* Special pack delivered on specific customer order. Please contact your local representative for more information.

† Larger quantities are available. Please contact your local representative for more information.

Related products	Quantity	Code No.
Glutathione Sepharose 4 Fast Flow	25 ml	17-5132-01
Glutathione Sepharose 4 Fast Flow	100 ml	17-5132-02
Glutathione Sepharose 4 Fast Flow	500 ml[1]	17-5132-03
GSTPrep™ FF 16/10	1 × 20 ml	17-5234-01
HiPrep™ 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml[2]	11-0003-29
PreScission Protease	500 units	27-0843-01
Thrombin	500 units	27-0846-01
Factor Xa	400 units	27-0849-01
GST Detection Module	50 reactions	27-4590-01
Anti-GST Antibody	0.5 ml	27-4577-01

[1] Larger quantities are available. Please contact your local representative for more information.

[2] Special pack size delivered on specific customer order. Please contact your local representative for more information.

Empty lab-scale columns	Quantity	Code No.
Tricorn 5/20 column	1	18-1163-08
Tricorn 5/50 column	1	18-1163-09
Tricorn 10/20 column	1	18-1163-13
Tricorn 10/50 column	1	18-1163-14
Tricorn 10/100 column	1	18-1163-15
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 no.
1/16" male/Luer female[1]	2	18-1112-51
Tubing connector flangeless/M6 female[1]	2	18-1003-68
Tubing connector flangeless/M6 male[1]	2	18-1017-98
Union 1/16" female/M6 male[1]	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"[2]	5	11-0004-64
Fingertight stop plug, 1/16"[3]	5	11-0003-55

[1] One connector included in each HiTrap package

[2] Two, five, or seven female stop plugs included in HiTrap packages, depending on products

[3] One fingertight stop plug is connected to the top of each HiTrap column

Related literature	Quantity	Code No.
GST Gene Fusion System Handbook	1	18-1157-58
Recombinant Protein Purification Handbook, Principles and Methods	1	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	1	18-1121-86
Convenient Protein Purification, HiTrap Column Guide	1	18-1129-81

www.gehealthcare.com/protein-purification
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