GSTrap 4B columns

GSTrap™ 4B columns are prepacked 1 ml and 5 ml HiTrap™ columns (Fig 1) for convenient, one-step purification of glutathione S-transferase (GST) tagged proteins, other glutathione S-transferases, and glutathione-binding proteins.
The columns are prepacked with Glutathione Sepharose™ 4B. The high binding capacity of GSTrap 4B columns complements the existing range of GSTrap FF and GSTrap HP columns, increasing the range of options available for purification of different GST-tagged proteins.

Fig 1. GSTrap 4B 1 ml and 5 ml columns are prepacked with Glutathione Sepharose 4B for efficient purification of GST-tagged proteins.

GSTrap 4B columns offer:

- Simple, one-step purification of GST-tagged proteins
- Prepacked columns with Glutathione Sepharose 4B for high reproducibility
- Simple operation using a syringe, pump, or chromatography system such as ÄKTA™ design

GST-tagged proteins expressed using, for example, pGEX vectors, can be purified directly from pretreated bacterial lysates with a one-step method on GSTrap 4B. Tagged proteins are eluted under mild, nondenaturing conditions that preserve protein antigenicity and function.

Chromatography medium characteristics

GSTrap 4B columns are delivered prepacked with Glutathione Sepharose 4B. The glutathione ligand is coupled via a 10-carbon linker to 4% agarose. Coupling is optimized to give a high binding capacity for GST-tagged proteins and other glutathione-binding proteins. Binding capacity of the medium is ≥ 10 mg GST-tagged protein/ml medium depending on size, conformation, and concentration of the protein in the sample loaded. The binding capacity also varies depending on the flow rate. The GST tag can be removed by treatment with an appropriate site-specific protease, such as PreScission™ Protease. Proteolytic cleavage can be performed while the tagged protein is bound to GSTrap 4B or, alternatively, after elution. Cleavage on GSTrap 4B eliminates the extra step of separating the released protein from GST, since the GST tag remains bound while the target protein is eluted using binding buffer.

Column characteristics

The column hardware of GSTrap 4B is composed of biocompatible polypropylene. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. The columns have porous top and bottom frits that allow high flow rates. Connectors for using the columns with a syringe, laboratory pump, or chromatography system such as ÄKTA design are included in each package. Note that the columns cannot be opened or repacked.

Table 1. Characteristics of GSTrap 4B columns

Column dimensions (i.d. × h)	0.7 × 2.5 cm (1 ml) and 1.6 × 2.5 cm (5 ml)
Column volumes	1 ml and 5 ml
Medium	Glutathione Sepharose 4B
Matrix	4% agarose
Mean particle size	90 µm
Ligand	Glutathione and 10-carbon linker arm
Ligand concentration	7 to 15 µmol glutathione/ml medium
Binding capacity[1]	≥ 10 mg recombinant GST-tagged protein (M_r 45 000)/ml medium
Maximum back pressure	3 bar (0.3 MPa)
Recommended flow rates[1]	Sample loading: 0.2 to 1.0 ml/min (1 ml) and 0.5 to 2.0 ml/min (5 ml); Washing and elution: 1 ml/min (1 ml) and 5 ml/min[2] (5 ml)
Chemical stability medium	All commonly used aqueous buffers, e.g. 1 M acetate, pH 4.0 and 6 M guanidine-HCl for 1 h at room temperature
pH stability	4 to 13
Storage	4°C to 30°C in 20% ethanol

[1] Binding of GST-tagged protein depends on size, conformation, and concentration of the protein in the sample loaded. Binding of GST to glutathione is also flow-dependent and lower flow rates during sample loading often increase the binding capacity. Protein characteristics, pH, and temperature may also affect the binding capacity.

[2] Recommended flow rate during washing and elution for GSTrap 4B 5 ml column at 4°C to 8°C is up to 4 ml/min.

Operation

GSTrap 4B columns are quick and easy to use with a syringe, pump, or chromatography system such as ÄKTA design. An application example where GSTrap 4B was used for automated purification of a GST-tagged protein on ÄKTAxpress™ is described later.

Glutathione Sepharose 4B is also available in 100 ml and 300 ml pack sizes.

Manual purification with GSTrap 4B columns is easily conducted with a syringe (connectors are provided). Figure 2 illustrates this technique.

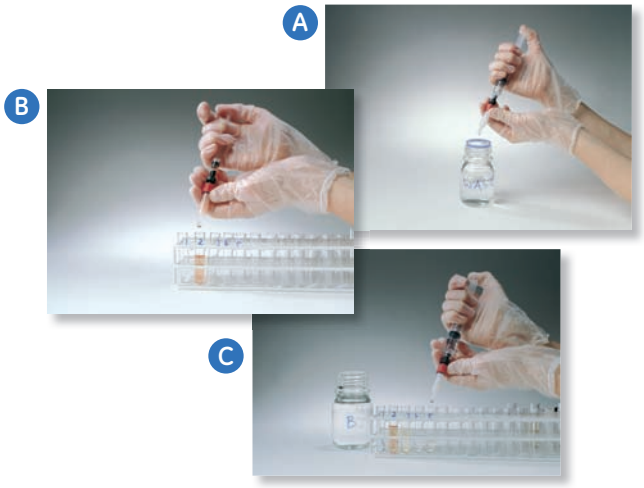


Fig 2. Using GSTrap 4B with a syringe. Prepare buffers and sample. Remove stop-plug from top of column and snap off the end. (A) Load sample and (B) collect fractions. (C) Wash, elute, and continue collecting fractions.

One-step purification of two different proteins using GSTrap 4B

The binding efficiency of GST-tagged proteins to GSTrap 4B depends on the characteristics and concentration of the protein in sample loaded to the column. To illustrate this, 12 ml of *E. coli* lysate containing GST-tagged hippocalcin or GST-tagged pura was applied to two separate GSTrap 4B 1 ml columns. Prior to loading on the columns, samples were subjected to enzymatic and mechanical lysis and clarified by centrifugation and filtration.

The purification of GST-hippocalcin and GST-pura is shown in Figure 3. Different shapes of the peaks of GST-hippocalcin and GST-pura in the chromatograms were obtained. SDS-PAGE of eluted target protein pools shows the purity of the target proteins purified in one step from the *E. coli* lysate.

Column:	GSTrap 4B, 1 ml
Sample:	Clarified *E. coli* lysates containing expressed GST-hippocalcin, M_r 45 000 or GST-pura M_r 58 000
Sample volume:	12 ml
Binding buffer:	10 mM sodium phosphate, 140 mM NaCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 10 mM glutathione, pH 8.0
Flow rate:	Sample loading, 0.3 ml/min Wash and elution, 1 ml/min
Running temperature:	22°C
System:	ÄKTAexplorer™ 100

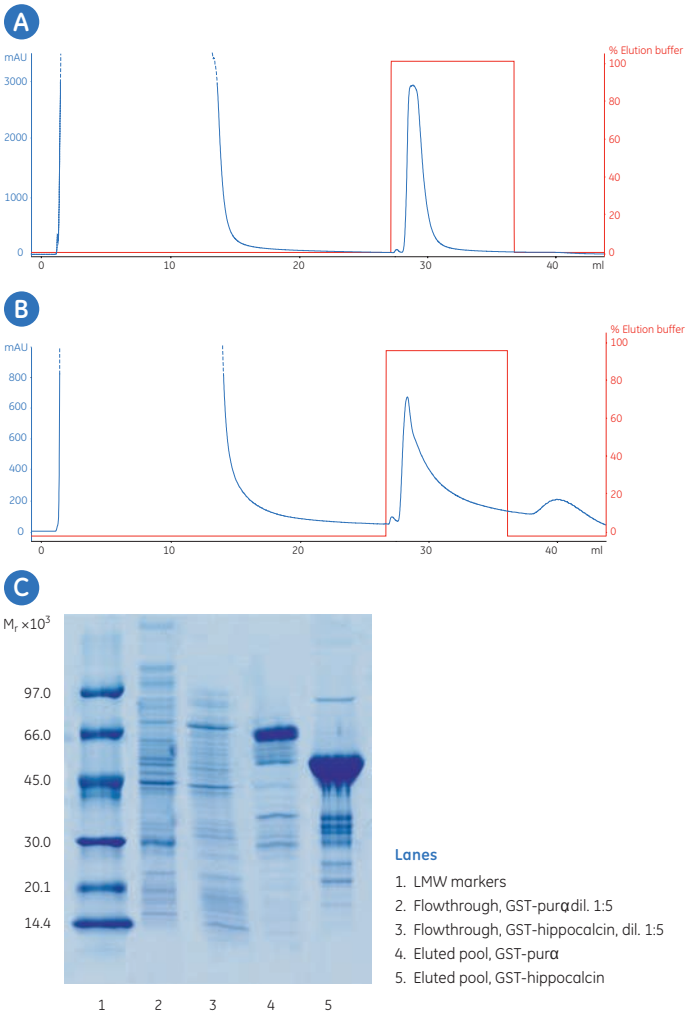


Fig 3. Purification of 12 ml volumes of *E. coli* lysate containing GST-hippocalcin or GST-pura (A) GST-hippocalcin. (B) GST-pura (C) SDS-PAGE (ExcelGel™ SDS Gradient 8–18) under reducing conditions shows the purified target proteins (lanes 4 and 5).

The yield of eluted target proteins, as estimated by absorbance measurement at 280 nm, was 15.3 mg for GST-hippocalcin and 13.7 mg for GST-puro on this 1 ml GSTrap 4B column.

Repeated purifications with high reproducibility

The reproducibility of repeated purifications using a GSTrap 4B 1 ml column was tested. Five repeated purifications of GST-tagged hippocalcin from clarified *E. coli* lysate were performed. The *E. coli* paste was lysed enzymatically, sonicated, and filtered before loading on the column.

Five repetitive purifications of GST-hippocalcin were performed on a GSTrap 4B 1 ml column; 5 ml samples were loaded in each run (Fig 4A). Reproducibility between purification runs was high. The yield of recovered protein was 10.1, 9.4, 9.3, 9.1, and 8.7 mg from the five purification runs, respectively.

SDS-PAGE showed that the purity of recovered GST-hippocalcin was not affected by the number of purification runs on the GSTrap 4B column (Fig 4B).

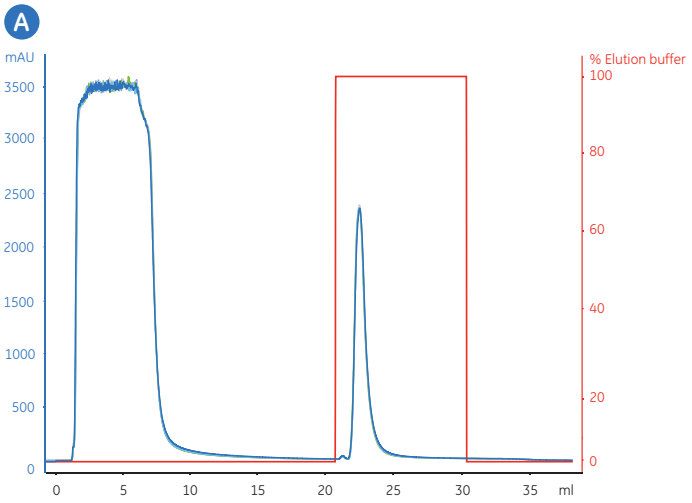
Scaling up purification from 1 ml to 5 ml GSTrap 4B columns

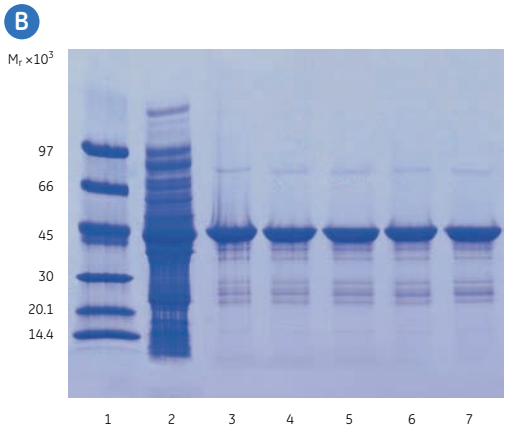
Purification of GST-tagged hippocalcin from clarified *E. coli* lysates was scaled-up from 1 ml to 5 ml GSTrap 4B columns. Lysis of *E. coli* containing GST-hippocalcin was performed enzymatically followed by sonication. The lysate was clarified by centrifugation and filtration; 5 ml and 25 ml of the clarified lysate was loaded on 1 ml and 5 ml GSTrap 4B columns, respectively.

Figure 5A and B shows the chromatograms from the two runs.

The amount of eluted protein, determined by measuring absorbance at 280 nm, was 9 mg after purification on the GSTrap 4B 1 ml and 46 mg after purification on the GSTrap 4B 5 ml column. Similar purity of eluted GST-hippocalcin was obtained from purifications on GSTrap 4B 1 ml and 5 ml columns (lanes 5 and 6, Fig 5C). The results show that the scale-up is highly consistent and does not significantly affect the recovery and purity of the target protein (Fig 5C).

Column:	GSTrap 4B, 1 ml
Sample:	Clarified *E. coli* lysate containing expressed GST-hippocalcin, M_r 45 000
Sample volume:	5 ml
Binding buffer:	10 mM sodium phosphate, 140 mM NaCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 20 mM glutathione, pH 8.0
Flow rate:	Sample loading, 0.3 ml/min Wash and elution, 1 ml/min
Running temperature:	22°C
System:	ÄKTAexplorer 100





Lanes

1. LMW markers
2. Start material, diluted 1:10
3. Run 1 eluted pool, diluted 1:4
4. Run 2 eluted pool, diluted 1:4
5. Run 3 eluted pool, diluted 1:4
6. Run 4 eluted pool, diluted 1:4
7. Run 5 eluted pool, diluted 1:4

Fig 4. Five repeated purification runs of GST-hippocalcin from *E. coli* lysate. (A) Absorbance curves (overlaid) at 280 nm for the five purification runs. (B) Reducing SDS-PAGE (ExcelGel SDS Gradient 8–18) of pools from the eluted peaks shows that purity of recovered target protein is not significantly affected by the number of purification runs (lanes 3 to 7).

Columns:	GSTrap 4B, 1 ml and 5 ml
Sample:	Clarified *E. coli* lysate containing expressed GST-hippocalcin, M_r 45 000
Sample volume:	5 ml and 25 ml on 1 ml and 5 ml columns, respectively
Binding buffer:	10 mM sodium phosphate, 140 mM NaCl, pH 7.4
Elution buffer:	50 mM Tris-HCl, 20 mM glutathione, pH 8.0
Flow rate, sample loading:	1 ml column, 0.3 ml/min 5 ml column, 1 ml/min
Flow rate, wash and elution:	1 ml column, 1 ml/min 5 ml column, 5 ml/min
Running temperature:	22°C
System:	ÄKTAexplorer 100

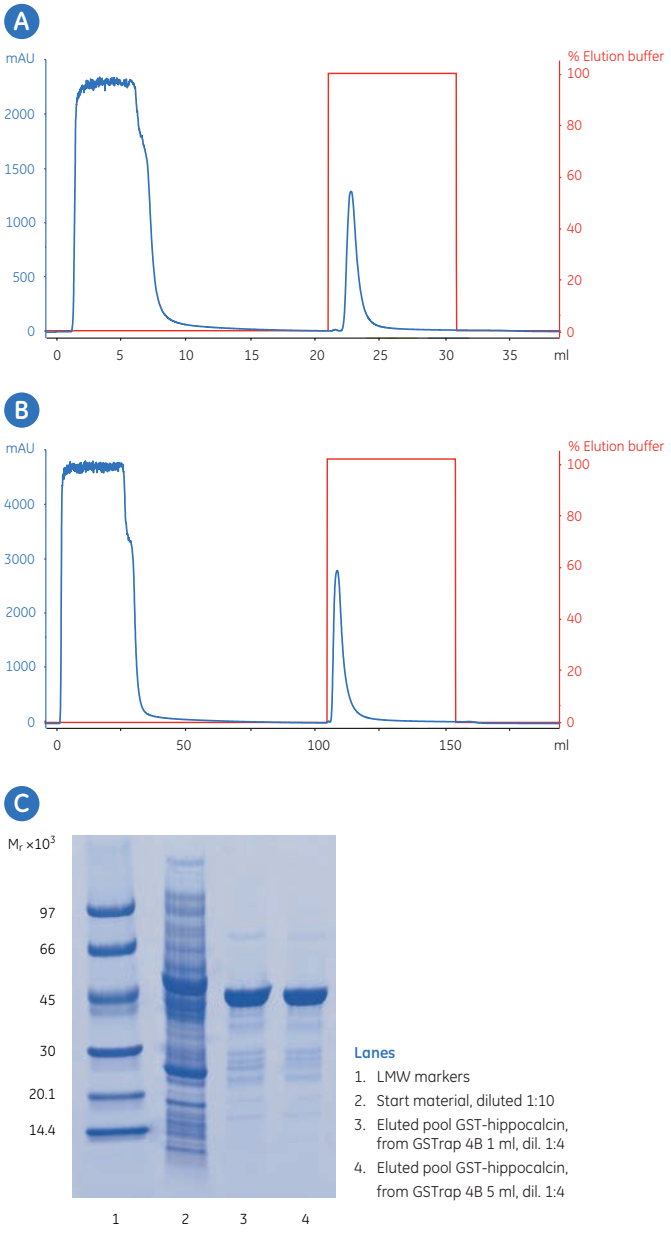


Fig 5. Scale-up purification of GST-hippocalcin from (A) a GSTrap 4B 1 ml to (B) GSTrap 4B 5 ml column. (C) SDS-PAGE (ExcelGel SDS Gradient 8–18%) confirms that scaling up from 1 ml to 5 ml GSTrap 4B columns does not affect the purification result.

Two-step, automated purification using ÄKTAxpress

A two-step, automated purification of GST-hippocalcin from clarified *E. coli* lysate was performed on ÄKTAxpress. A GSTrap 4B 1 ml column was used in the first affinity chromatography (AC) capture step and a HiLoad™ 16/60 Superdex™ 200 pg column for the polishing step using gel filtration.

Reducing agent (DTT) was included in both sample and buffers. ÄKTAxpress enabled automated loading of eluted fractions of the target protein from the capture step (GSTrap 4B) onto the gel filtration column.

Lysis of *E. coli* containing GST-hippocalcin was performed enzymatically followed by sonication. The lysate was clarified by centrifugation and filtration, and 5 ml of the clarified lysate was loaded on the 1 ml GSTrap 4B column. Chromatograms from the automated two-step purification, as well as SDS-PAGE of the eluted pool of target protein are shown in Figure 6. Two peaks were obtained after gel filtration: one small and one large. According to SDS-PAGE (only the pool of the large peak is shown, Fig 6B), both peaks contained GST-hippocalcin. From evaluation of the gel filtration step, the large peak seemed to be the dimer of GST-hippocalcin. The small peak is possibly a larger aggregate of GST-hippocalcin. The purity of the GST-hippocalcin was good (Fig 6C).

Yield of eluted GST-hippocalcin, determined by absorbance at 280 nm (calculated using UNICORN™ software), was 6.4 mg.

This application shows the benefit of using a two-step purification for increasing the purity of GST-hippocalcin. When comparing the results for a one-step purification (Fig 3B, lane 5) with this two-step purification (Fig 6C, lane 4), an increased purity of the GST-hippocalcin target protein was observed.

Storage

GSTrap 4B columns should be stored in 20% ethanol at 4°C to 30°C.

Column:	GSTrap 4B, 1 ml HiLoad 16/60 Superdex 200 pg, 120 ml
Sample:	Clarified *E. coli* lysate containing expressed GST-hippocalcin, M, 45 000
Sample volume:	5 ml (GSTrap 4B)
Binding buffer:	10 mM sodium phosphate, 140 mM NaCl, 20 mM DTT, pH 7.4
Elution buffer:	50 mM Tris-HCl, 20 mM glutathione, 20 mM DTT, pH 8.0
Buffer gel filtration:	10 mM sodium phosphate, 140 mM NaCl, 20 mM DTT, pH 7.4
Flow rate:	Sample loading, 0.3 ml/min (GSTrap 4B) Wash and elution, 1 ml/min (GSTrap 4B) 1.5 ml/min (HiLoad 16/60 Superdex 200 pg)
Running temperature:	22°C
System:	ÄKTAxpress

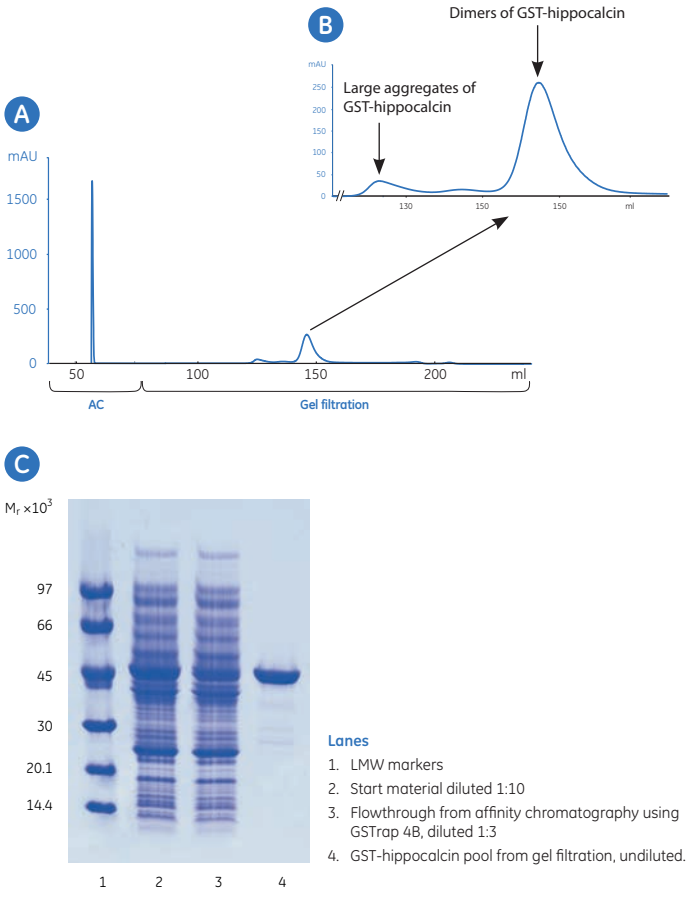


Fig 6. (A). Purification of GST-hippocalcin from *E. coli* lysate using an automated two-step purification on ÄKTAxpress. (B) Enlargement of the peak from the gel filtration step revealed large aggregates and dimers of purified GST-hippocalcin. (C) SDS-PAGE (ExcelGel SDS Gradient 8%–18%) showing final purity of GST-hippocalcin (lane 4).

Ordering information

Product[1]	Quantity	Code No.
GSTrap 4B	5 × 1 ml	28-4017-45
	100 × 1 ml[2]	28-4017-46
	1 × 5 ml	28-4017-47
	5 × 5 ml	28-4017-48
	100 × 5 ml[2]	28-4017-49

[1] All columns include connectors for easy connection to a syringe, pump, or chromatography system

[2] Pack size available by specific customer order

Related products	Quantity	Code No.
Glutathione Sepharose 4B	10 ml	17-0756-01
	100 ml	27-4574-01
	300 ml	17-0756-04
HiTrap Benzamidine FF (high sub)	5 × 1 ml	17-5143-01
	2 × 1 ml	17-5143-02
	1 × 5 ml	17-5144-01
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml[1]	11-0003-29
HiPrep™ 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
GST Detection Module	50 reactions	27-4590-01
Glutathione S-transferase gene fusion vectors (pGEX vectors)[2]	Various	Various
Anti-GST Antibody	0.5 ml	27-4577-01

[1] Pack size available by specific customer order

[2] All pGEX vectors include *E. coli* BL21 cells. Contact GE Healthcare for more information

Site-specific proteases	Quantity	Code No.
PreScission Protease	500 units	27-0843-01
Thrombin	500 units	27-0846-01
Factor Xa	400 units	27-0849-01

Accessories	Quantity	Code No.
1/16" male/Luer female[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[1]	5	18-3858-01
Union Luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16"[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 stop plugs female included in HiTrap packages depending on the product

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

Literature	Code No.
GST Gene Fusion System Handbook	18-1157-58
Recombinant Protein Handbook, Methods and Principles	18-1142-75
Affinity Chromatography Handbook, Methods and Principles	18-1022-29
Glutathione Sepharose, Selection Guide	28-9168-33
HiTrap Column Guide	18-1129-81
Prepacked chromatography columns for ÄKTA design systems, Selection Guide	28-9317-78

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