Dextrin **Sepharose** High Performance **MBPTrap** HP

AFFINITY PURIFICATION

Dextrin Sepharose™ High Performance is a chromatography medium for purifying recombinant proteins tagged with maltose binding protein (MBP). The chromatography medium is available in 25 ml and 100 ml lab packs and prepacked in 1 ml and 5 ml MBPTrap™ HP columns.

Tagging proteins with MBP often gives increased expression levels and higher solubility of the target protein. Proper folding of the attached protein has also been shown to be promoted by the MBP tag. Since MBP increases solubility, the tag is particularly useful for recombinant proteins accumulated in an insoluble form (inclusion bodies).

Affinity purification using Dextrin Sepharose High Performance takes place under physiological conditions and mild elution is performed using maltose . This preserves the activity of the target protein. Even intact protein complexes may be purified. In addition, the high specificity of the binding means that very high purity can be achieved in just one step in combination with high binding capacity.

Dextrin Sepharose High Performance benefits:

- Highly pure MBP-tagged recombinant proteins eluted in concentrated form and small volumes
- Physiological conditions and mild elution preserve target protein activity
- Compatible with commonly used aqueous buffers and easily regenerated using 0.5 M NaOH
- Easy scale-up
- Prepacked MBPTrap HP 1 ml and 5 ml columns offer convenience, save time, and ensure reproducible results

Fig 1. Dextrin Sepharose High Performance, also prepacked as MBPTrap HP columns, give fast and convenient affinity purifications of recombinant proteins tagged with maltose binding protein.

Description

Chromatography medium characteristics

Dextrin Sepharose High Performance is a robust, high-resolution medium based on the 34 µm Sepharose High Performance matrix. The small, evenly sized beads ensure that MBP-tagged proteins elute in narrow peaks, thus minimizing the need for further concentration steps. Dextrin Sepharose High Performance tolerates all commonly used aqueous buffers and is easily regenerated using 0.5 M NaOH allowing the same column to beused for repeated purifications. Table 1 summarizes the characteristics of Dextrin Sepharose High Performance.

MBPTrap HP column characteristics

These 1 ml and 5 ml columns are made of biocompatible polypropylene that does not interact with biomolecules. Prepacked MBPTrap HP columns provide fast, simple and easy separations in a convenient format. They are delivered with a stopper on the inlet and a snap-off end on the outlet. Porous top and bottom frits allow high flow rates. MBPTrap HP columns belong to the HiTrap™ family of prepacked columns.

Note that HiTrap columns cannot be opened or refilled. Table 2 summarizes the characteristics of prepacked MBPTrap HP columns.

Table 1. Characteristics of Dextrin Sepharose High Performance

Matrix	Rigid, highly cross-linked 6% agarose
Average particle size	34 µm
Ligand	Dextrin
Dynamic binding capacity[1]	Approx. 7 mg/ml medium MBP2*-paramyosin-δ-Sal (M_r ~70 000, multimer in solution)
	Approx. 16 mg/ml medium MBP2*-β-galactosidase (M_r ~158 000, multimer in solution)
Recommended flow rate[2]	≤ 150 cm/h
Maximum linear flow rate[2]	< 300 cm/h
Maximum back pressure[2]	0.3 MPa, 3 bar, 43.5 psi
Chemical stability[3]	Stable in all commonly used aqueous buffers, 0.5 M NaOH (regeneration and cleaning)
pH stability	
Working[4]	> 7
Cleaning[5]	2 to 13
Storage	4°C to 8°C in 20% ethanol

[1] Binding capacity is protein dependent
[2] H_2O at room temperature
[3] The presence of reducing agents, e.g., 5 mM DTT, may decrease yield. Higher ionic strength does not decrease affinity since MBP binds to dextrin primarily by hydrogen binding. Agents that interfere with hydrogen binding, such as urea and guanidine hydrochloride, are not recommended. The presence of 10% glycerol may decrease the yield and 0.1% SDS completely eliminates the binding
[4] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance
[5] Refers to the pH interval for regeneration

Table 2. Characteristics of MBPTrap HP

Column volume	1 ml or 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml), 1.6 × 2.5 cm (5 ml)
Recommended flow rates[1]	1 and 5 ml/min for 1 and 5 ml columns, respectively
Maximum flow rates[1]	4 and 20 ml/min for 1 and 5 ml columns, respectively
Column hardware pressure limit	0.5 MPa, 5 bar, 70 psi

[1] H_2O at room temperature

Use and applications

Packing in laboratory columns

Dextrin Sepharose High Performance is supplied pre-swollen in 25 ml and 100 ml packs. The medium is easy to pack and use in, for example, laboratory columns from the Tricorn™ and XK series (see *Ordering information*). Full user instructions are supplied with each pack.

MBPTrap HP columns

Purifications on MBPTrap HP 1 ml and 5 ml columns are easily performed using a syringe and the provided Luer adapter, a laboratory pump, or a chromatography system such as an ÄKTA™ system. The columns are ideal for automated purification in combination with another chromatography step, for example, gel filtration or another affinity chromatography step. Their use is also facilitated by simple, time-saving operation, easy scale up, and fast and effective regeneration.

Two-step purification on ÄKTAxpress

MBP2*-paramyosin-δ-Sal (M_r ~70 000), a multimer in solution, was purified from *E. coli* lysate using a two-step protocol with ÄKTAxpress. MBPTrap HP 1 ml was used as the first affinity chromatography (AC) step in an automated two-step purification run. The second step, gel filtration (GF), was run on HiLoad™ 16/60 Superdex™ 200 pg. Figure 2 shows the running conditions and the resulting chromatogram of the automated purification.

AC column:	MBPTrap HP 1 ml
Sample:	MBP2*-paramyosin-δ-Sal (M_r ~70 000) in *E. coli* lysate
Sample volume:	7 ml
Binding buffer:	20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer:	10 mM maltose in binding buffer
Flow rate:	1.0 ml/min (0.5 ml/min during sample application)
System:	ÄKTAxpress
GF column:	HiLoad 16/60 Superdex 200 pg
Sample:	Eluted pool from MBPTrap HP 1 ml
Buffer:	10 mM sodium phosphate, 140 mM NaCl, pH 7.4
Flow rate:	1.5 ml/min
System:	ÄKTAxpress

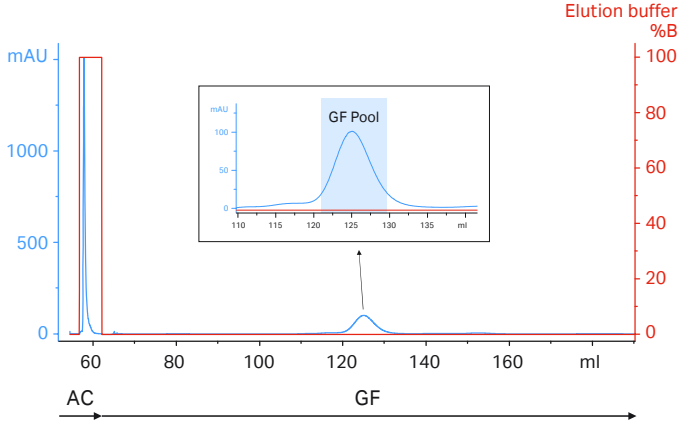
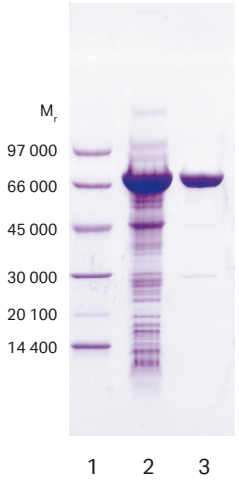


Fig 2. Automated purification of MBP2*-paramyosin-δ-Sal using a two-step AC-GF protocol on MBPTrap HP 1 ml (AC) and HiLoad 16/60 Superdex 200 pg (GF).

Total final yield after the two steps was 2.2 mg and the overall run time was only 3.4 h. The SDS-PAGE analysis in Figure 3 shows the high purity of the pooled fraction from the final gel filtration step.



Lane
1. Low molecular weight markers
2. Start material, MBP2*-paramyosin-δ-Sal in *E. coli* lysate
3. Final eluted gel filtration pool

Fig 3. SDS-PAGE analysis (reduced conditions) of the purification of MBP2*-paramyosin-δ-Sal.

Simplified purification of a protein involved in metabolic disease

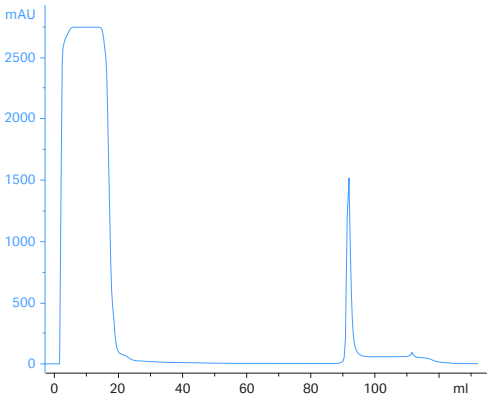
Using the MBPTrap HP column eliminated a concentration step in a purification procedure for medium-chain acyl- CoA dehydrogenase (MCAD). This M_r 85 500 homotetramer, which is involved in metabolic disease, was purified for stability, folding, and kinetic studies. MBPTrap HP 5 ml replaced the affinity chromatography step used in the original protocol. The target protein eluted from the MBPTrap HP column was highly concentrated and in a small volume, subsequently, the concentration step prior to final gel filtration used in the original protocol could be avoided.

The purity of the eluted fractions from MBPTrap HP and gel filtration was determined by SDS-PAGE analysis. As well as the target protein, some additional proteins were detected after the affinity step. This may be due to the presence of truncated variants still having the N-terminal MBP tag intact, or possibly *E. coli* proteins associated with the target protein (this was not evaluated further). Final purity after gel filtration was high (greater than 95%) according to SDS-PAGE analysis. Final yield of MCAD was approximately 8.4 mg. As well as cutting total purification time and eliminating the concentration step, the recovery of target protein was also increased due to fewer handling steps being needed.

Figure 4 shows both chromatograms and Figure 5 the SDS-PAGE analysis of the eluted fractions.

Column: MBPTrap HP 5 ml
Sample: N-terminal MBP-MCAD in *E. coli* lysate
Sample volume: 15 ml
Binding buffer: 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer: 10 mM maltose in binding buffer
Flow rate: 5.0 ml/min (0.5 ml/min during sample loading)
System: ÄKTAprime

(A)



Column: Superdex 200 pg in XK 16/20
Sample: Eluted fraction from MBPTrap HP 5 ml
Sample volume: 2 ml
Buffer: 20 mM HEPES, 200 mM NaCl, pH 7.0
Flow rate: 0.4 ml/min
System: ÄKTAprime

(B)

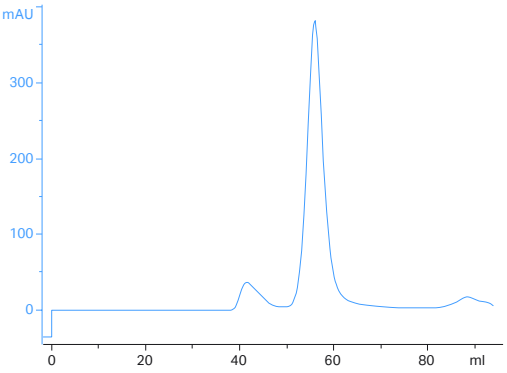
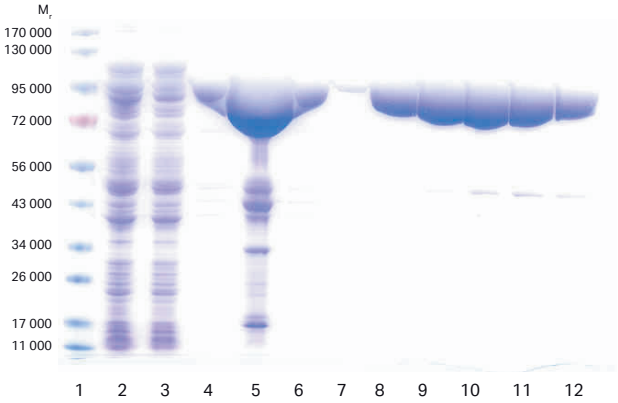


Fig 4. Purification of MCAD on (A) MBPTrap HP followed by (B) Superdex 200 pg.



Lane
1. Molecular weight markers
2. Start material, N-terminal MBP-MCAD in *E. coli* lysate, dil. 1:6
3. Flowthrough MBPTrap HP, dil. 1:6
4–6. Eluted fractions from MBPTrap HP
7–12. Eluted fractions from gel filtration

Fig 5. SDS-PAGE analysis (reduced conditions) of fractions from the two-step purification of MCAD.

Scaling up

Scale-up can be achieved by increasing the bed volume while keeping the residence time constant. This approach maintains chromatographic performance during scale-up.

MBP2*-β-galactosidase (M_r ~158 000), a recombinant tagged multimer, was purified on an MBPTrap HP 1 ml column on ÄKTAexplorer. The purification was scaled up to an MBPTrap HP 5 ml column followed by further scale-up to an XK 26/20 column packed with Dextrin Sepharose High Performance. The protein load was increased five-fold in each step (~10, ~50, and ~250 mg, respectively) and the residence time was ~2 min for all three columns.

Figure 6 shows running conditions for all runs and the chromatograms from the MBPTrap HP 1 ml and Dextrin Sepharose High Performance XK 26/20 runs. Figure 7 shows the SDS-PAGE results.

Columns:	MBPTrap HP 1 ml MBPTrap HP 5 ml Dextrin Sepharose High Performance packed in XK 26/20, 29 ml, bed height 5.5 cm
Sample:	MBP2*-β-galactosidase (M_r ~158 000) in *E. coli* lysate
Sample volumes:	5 ml (MBPTrap HP 1 ml) 25 ml (MBPTrap HP 5 ml), 125 ml (XK 26/20 column)
Binding buffer:	20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer:	10 mM maltose in binding buffer
Flow rates:	MBPTrap HP 1 ml: 1.0 ml/min (0.5 ml/min during sample loading) MBPTrap HP 5 ml: 5.0 ml/min (2.5 ml/min during sample loading) XK 26/20 column: 13 ml/min
System:	ÄKTAexplorer

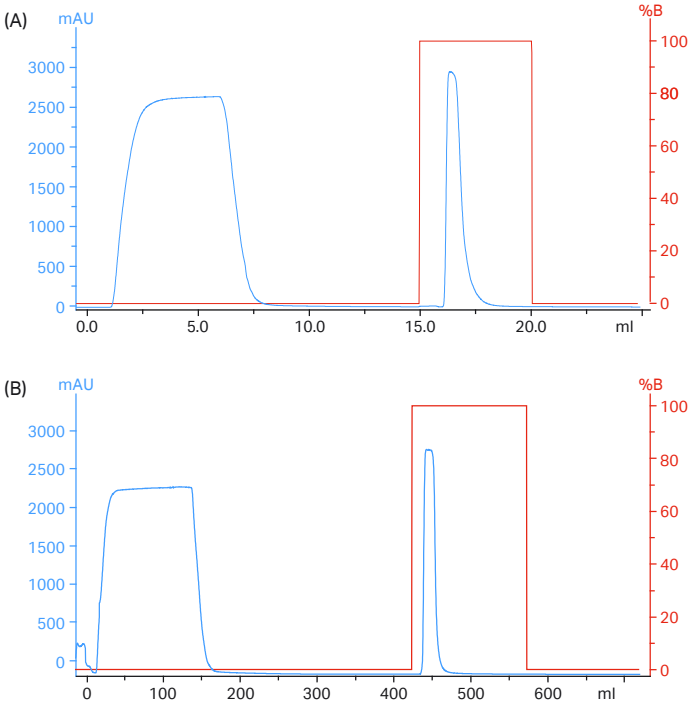
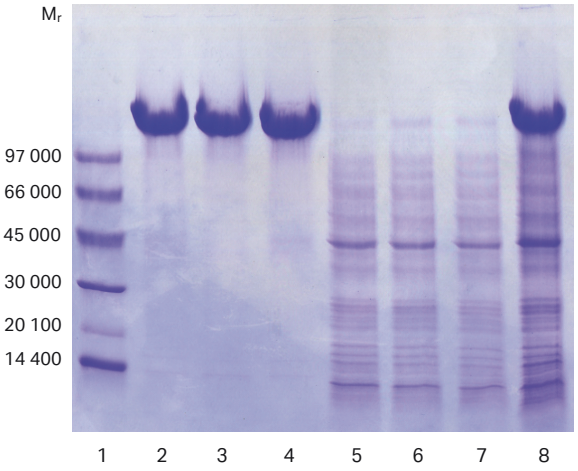


Fig 6. Scale-up of MBP2*-β-galactosidase purification, (A) MBPTrap HP 1 ml (B) Dextrin Sepharose High Performance XK 26/20.



Lane
1. Low molecular weight markers
2. Eluted pool, MBPTrap HP 1 ml, dil. 1:6
3. Eluted pool, MBPTrap HP 5 ml, dil. 1:12
4. Eluted pool, XK 26/20, dil. 1:12
5. Flowthrough, MBPTrap HP 1 ml, dil. 1:3
6. Flowthrough, MBPTrap HP 5 ml, dil. 1:3
7. Flowthrough, XK 26/20, dil. 1:3

Fig 7. SDS-PAGE analysis (reduced conditions) of the scale-up study.

The columns gave comparable results with high purity and similar yields (approx. 60%, Table 3), confirming the ease and reproducibility of scaling up purifications from MBPTrap HP columns to XK 26/20 column.

An alternative method for quick scale-up is to connect two or three MBPTrap HP columns in series, but this may increase backpressure.

Table 3. Yield calculated in milligram and percent

Column	Yield (mg)	Yield (%)
MBPTrap HP 1 ml	6.4	64
MBPTrap HP 5 ml	29.5	59
XK 26/20 packed with Dextrin Sepharose High Performance, 29 ml	141.4	57

Regeneration with NaOH

Repeated purifications run on the same MBPTrap HP column without regeneration may gradually decrease recovery. Regular regeneration, however, allows the same column to be run many times with retained performance, thus promoting cost-effective use. Regenerating MBPTrap HP with 0.5 M NaOH is highly effective, as the following study demonstrates. MBP2*-β-galactosidase in *E. coli* lysate was purified six times on the same MBPTrap HP 1 ml. Regeneration following each purification was performed using 1.5 M NaCl and 0.5 M NaOH (note that sodium chloride is often not necessary and may be omitted).

Figure 8 shows the six repeated purification runs and illustrates the very high reproducibility and yield possible with MBPTrap HP columns. The high purity for each run was confirmed by SDS-PAGE analysis (Fig 9). Furthermore, the recovery remained constant throughout the entire study (Fig 10), thus demonstrating the benefit of regeneration with 0.5 M NaOH.

Column:	MBPTrap HP 1 ml
Sample:	MBP2*-β-galactosidase in *E. coli* lysate
Binding buffer:	20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer:	10 mM maltose in binding buffer
Regeneration:	3 ml 1.5 M NaCl, 3 ml distilled water, 3 ml 0.5 M NaOH, 3 ml distilled water
Flow rate:	1 ml/min (0.5 ml/min for sample loading and 0.5 M NaOH)
System:	ÄKTAexplorer

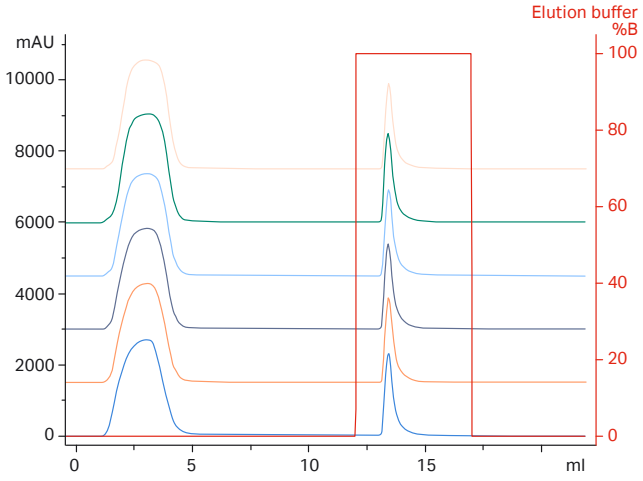
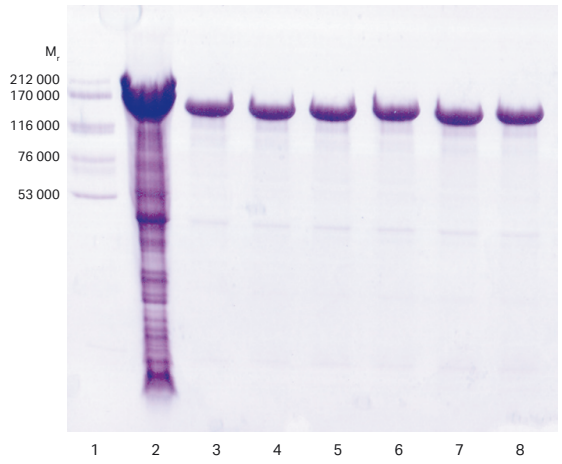


Fig 8. Six repeated purification runs including regeneration on the same MBPTrap HP 1 ml column.



Lane
1. High molecular weight markers
2. Start material, MBP2*-β-galactosidase in *E coli* lysate
3. Run 1, eluted pool
4. Run 2, eluted pool
5. Run 3, eluted pool
6. Run 4, eluted pool
7. Run 5, eluted pool
8. Run 6, eluted pool

Fig 9. SDS-PAGE analysis (reduced conditions) of the regeneration study indicates retained chromatographic performance and excellent reproducibility.

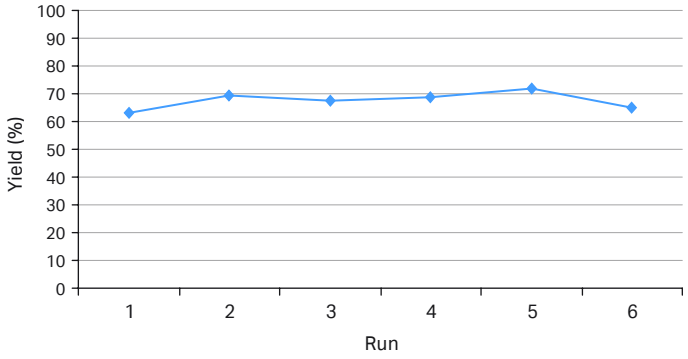


Fig 10. The yield in the eluted pools was retained over the course of the study, which comprised six standard purifications and five intermittent regenerations using 0.5 M NaOH.

Acknowledgement

We thank Dr. Esther M. Maier, Dr. von Haunersches Kinderspital, Munich, Germany, for fruitful discussions and excellent application work.

Ordering information

Product	Quantity	Code number
Dextrin Sepharose High Performance	25ml	28-9355-97
	100 ml	28-9355-98
MBPTrap HP	1 × 1 ml	29-0486-41
	5 × 1 ml	28-9187-78
	1 × 5 m	28-9187-79
	5 × 5 m	28-9187-80

Related products	Quantity	Code number
HiLoad 16/60 Superdex 200 pg	1 × 120 ml	17-1069-01
HiLoad 26/60 Superdex 200 pg	1 × 320 ml	17-1071-01

Empty lab-scale columns	Quantity	Code number
Tricorn 5/20 column, 5 mm i.d.	1	18-1163-08
Tricorn 5/50 column, 5 mm i.d.	1	18-1163-09
Tricorn 10/20 column, 10 mm i.d.	1	18-1163-13
Tricorn 10/50 column, 10 mm i.d.	1	18-1163-14
Tricorn 10/100 column, 10 mm i.d.	1	18-1163-15
XK 16/20 column, 16 mm i.d.	1	18-8773-01
XK 26/20 column, 26 mm i.d.	1	18-1000-72

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
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86
HiTrap Column Guide	18-1129-81
Prepacked chromatography columns for ÄKTA systems	28-9317-78

cytiva.com/hitrap
cytiva.com/protein-purification

CY13575-30Jul20-DF

cytiva