MBPTrap HP 1 ml and 5 ml

MBPTrap™ HP is a ready to use HiTrap™ column for purifying recombinant proteins tagged with maltose binding protein (MBP). The column is packed with Dextrin Sepharose™ High Performance based on a 34 µm bead-sized matrix. This gives a robust affinity medium with high resolution resulting in sharp, concentrated peaks, often making further concentration of the eluted target protein unnecessary.

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).

The affinity purification using MBPTrap HP takes place under physiological conditions, and mild elution by adding maltose 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.

MBPTrap HP columns 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
- Prepacked 1 ml and 5 ml format gives convenient, time-saving operation and reproducible results
- Easy scale up
- Simple operation with a syringe, peristaltic pump or chromatography system such as ÄKTAdesign™

Fig 1. MBPTrap HP 1 ml and 5 ml columns give fast and convenient affinity purifications of recombinant proteins tagged with maltose binding protein.

Description

Medium characteristics

MBPTrap HP 1 ml and 5 ml columns are prepacked with Dextrin Sepharose High Performance, 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. MBPTrap HP tolerates all commonly-used aqueous buffers and is easily regenerated using 0.5 M NaOH allowing the same column to be used for repeated purifications.

Column characteristics

The 1 ml and 5 ml columns are made of biocompatible polypropylene that does not interact with biomolecules. 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. Note that HiTrap columns cannot be opened or refilled. Table 1 summarizes the characteristics of prepacked MBPTrap HP columns.

Table 1. Characteristics of MBPTrap HP

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)
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[2]	1 and 5 ml/min for 1 and 5 ml columns respectively
Maximum flow rates[2]	4 and 20 ml/min for 1 and 5 ml columns respectively
Maximum back pressure[2]	0.3 MPa, 3 bar
Chemical stability[3]	Stable in all commonly used aqueous buffers
pH stability, (working range)	> 7
(short-term)	2 to 13
Storage	4 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

Use and applications

General

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

Two-step purification on ÄKTAxpress

MBPTrap HP 1 ml was used as the first affinity step in an automated two-step purification run on ÄKTAxpress™. The second step, gel filtration, was run on HiLoad™ 16/60 Superdex™ 200 pg. MBP2*-paramyosin- δ-Sal (M_r ~70 000), which exists as a multimer in solution, was purified from *E. coli* lysate. Figure 2 shows the running conditions and the resulting chromatogram of the automated purification. Total final yield after the two steps was 2.16 mg and the overall run time was only 3.4 hours. The SDS-PAGE analysis in Figure 3 shows the high purity of the pooled fraction from the final gel filtration step.

AC column: MBPTrap HP 1 ml
Sample: MBP2*-paramyosin-δ-Sal (M_r ~70 000) in *E. coli* lysate
Sample volume: 7 ml
Flow rate: 1.0 ml/min (0.5 ml/min during sample application)
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
System: ÄKTAxpress

GF column: HiLoad 16/60 Superdex 200 pg
Sample: Eluted pool from MBPTrap HP 1 ml
Flow rate: 1.5 ml/min
Buffer: 10 mM sodium phosphate, 140 mM NaCl, pH 7.4

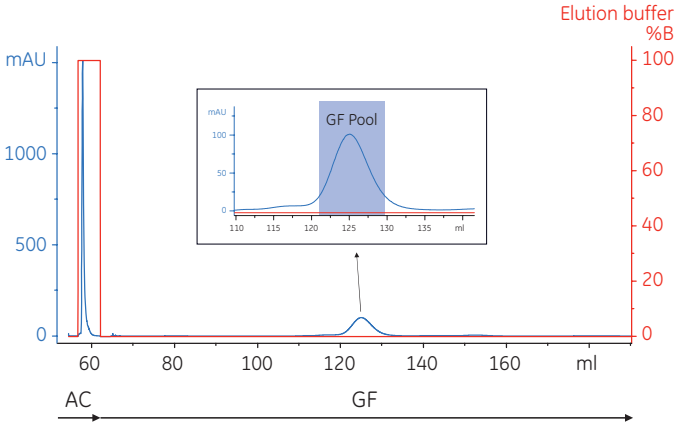
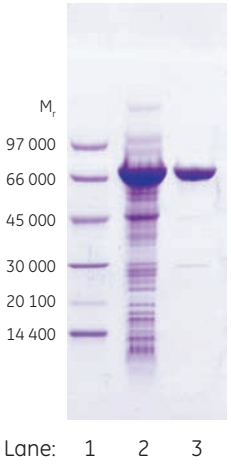


Fig 2. Automated purification of MBP2*-paramyosin-δ-Sal using the two-step AC-GF protocol on MBPTrap HP 1 ml (AC) and HiLoad 16/60 Superdex 200 pg (GF).



Lane
1 Low molecular weight marker, LMW
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.

Column:	MBPTrap HP 5 ml
Sample:	N-terminal MBP-MCAD in *E. coli* lysate
Sample volume:	15 ml
Flow rate:	5.0 ml/min (0.5 ml/min during sample loading)
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
System:	ÄKTAprime™

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

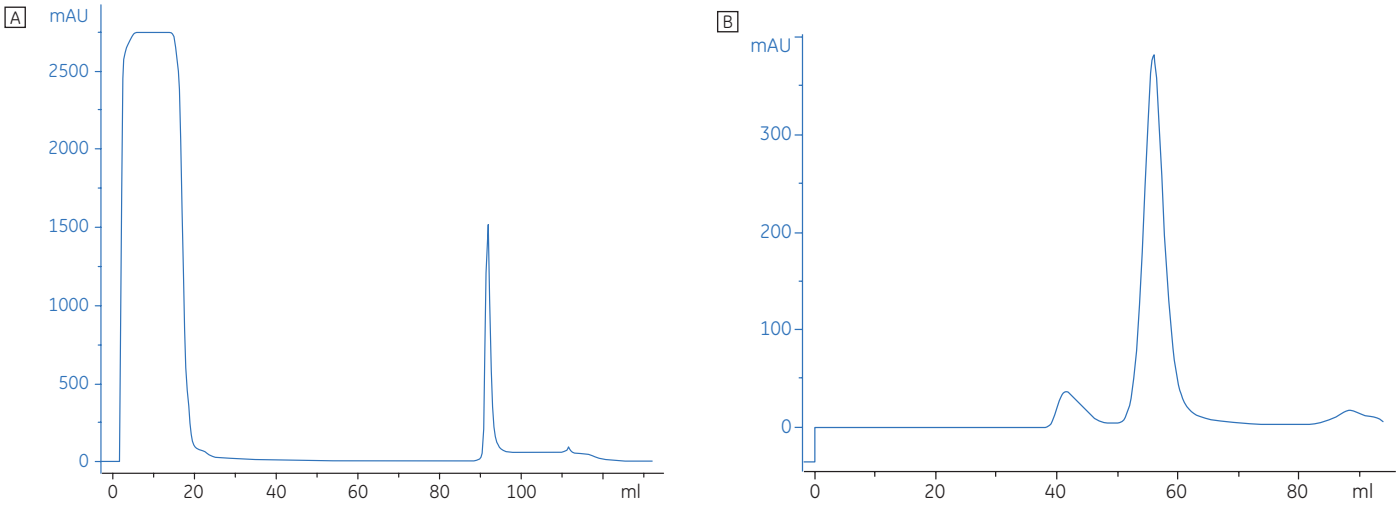
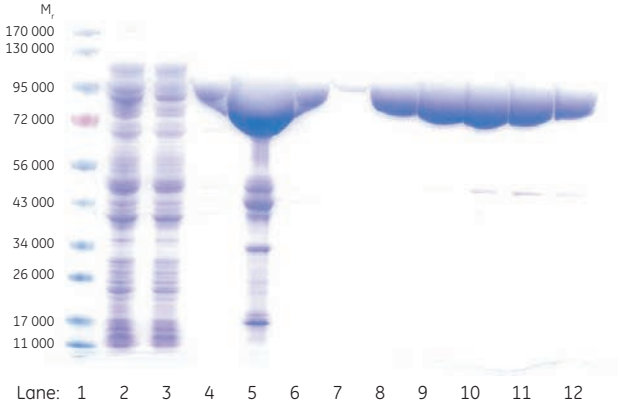


Fig 4. Purification of MCAD on **(A)** MBPTrap HP followed by **(B)** Superdex 200 pg.

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 earlier chromatography affinity step. The target protein eluted from the MBPTrap HP column was highly concentrated and in a small volume, subsequently, the former concentration step prior to final gel filtration 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 was approximately 8.4 mg MCAD. 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.



Lane	
1	Molecular weight marker
2	Start material, N-terminal MBP-MCAD in *E. coli* lysate, dil. 6×
3	Flow through MBPTrap HP, dil. 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.

Scale up from 1 ml to 5 ml MBPTrap HP columns

Increasing sample load and flow rate five-fold is the simplest way to scale up purifications from MBPTrap HP 1 ml to 5 ml columns. (An alternative method for quick scale-up is to connect two or three MBPTrap HP columns in series, but this will increase back-pressure.)

MBP2*-β-galactosidase (M_r ~158 000), a multimer in solution, was first purified on a MBPTrap HP 1 ml column on ÄKTAexplorer™ and then scaled up to the 5 ml column. Loadings were 3.6 mg and 18 mg MBP2*-β-galactosidase, respectively. The sample volumes applied were 2 ml for the 1 ml column and 10 ml for the 5 ml column.

Figure 6 shows running conditions and chromatograms, and Figure 7 the SDS-PAGE results. Similar purities (96.9% and 97.4 % respectively) and yields (62% and 63.5%) were obtained for both runs, confirming the ease and reproducibility of scaling up purifications from 1 ml to 5 ml MBPTrap HP columns.

Columns:	MBPTrap HP 1 ml MBPTrap HP 5 ml
Sample:	MBP2*-β-galactosidase (M_r ~158 000) in *E. coli* lysate
Sample volumes:	2 ml (1 ml column) 10 ml (5 ml column)
Flow rates:	1.0 ml/min (0.5 ml/min during sample loading and wash) (1 ml column) 5.0 ml/min (2.5 ml/min during sample loading and wash) (5 ml 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
System:	ÄKTAexplorer

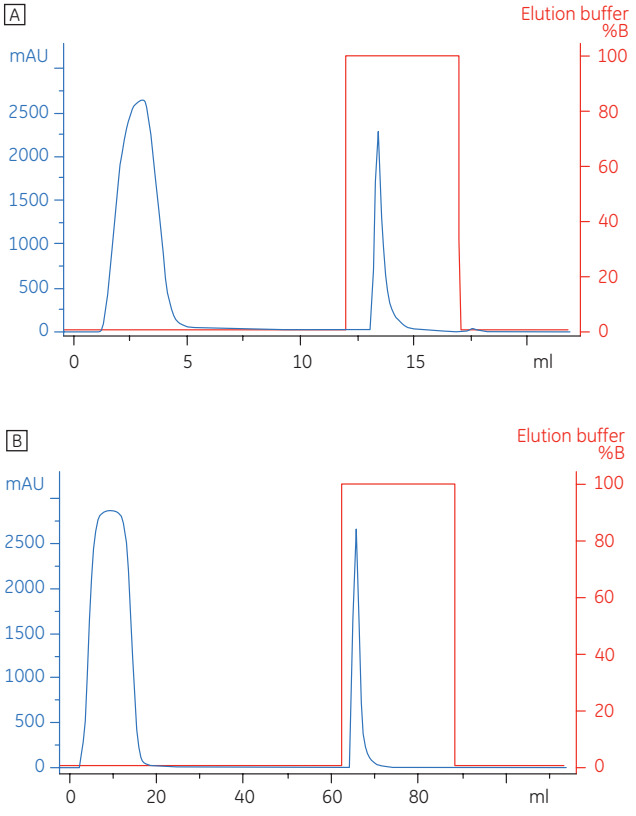
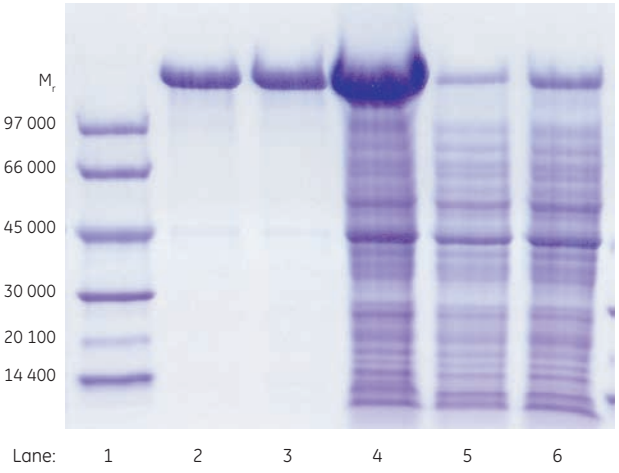


Fig 6. Scale up of MBP2*-β-galactosidase purification, **(A)** MBPTrap HP 1 ml **(B)** MBPTrap HP 5 ml.



Lane

1 Low molecular weight marker, LMW
2 Eluted pool, MBPTrap HP 1 ml
3 Eluted pool, MBPTrap HP 5 ml
4 Start material, MBP2*-β-galactosidase in *E. coli* lysate
5 Flow through, MBPTrap HP 1ml
6 Flow through, MBPTrap HP 5 ml

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

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 illustrate very high reproducibility and yield. 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 excellent effect of the 0.5 M NaOH regeneration protocol.

Column:	MBPTrap HP 1 ml
Sample:	MBP2*-β-galactosidase in *E. coli* lysate
Flow rate:	1 ml/min (0.5 ml/min for sample loading and 0.5 M NaOH)
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
System:	ÄKTAexplorer

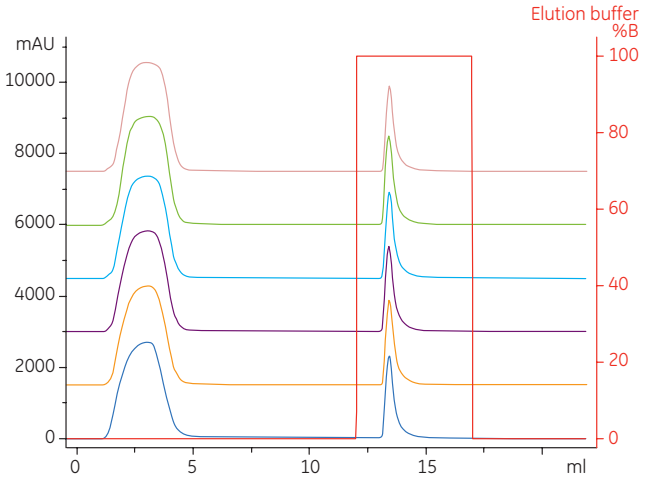
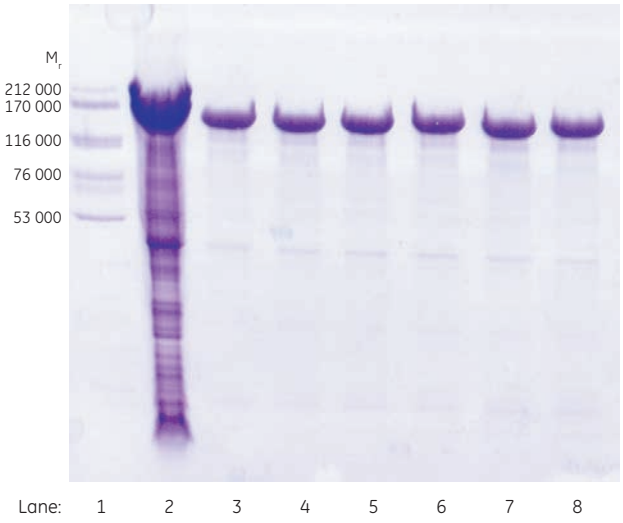


Fig 8. Six repeated purification runs including regeneration on the same MBPTrap HP 1 ml column.



Lane	
1	High molecular weight marker, HMW
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.

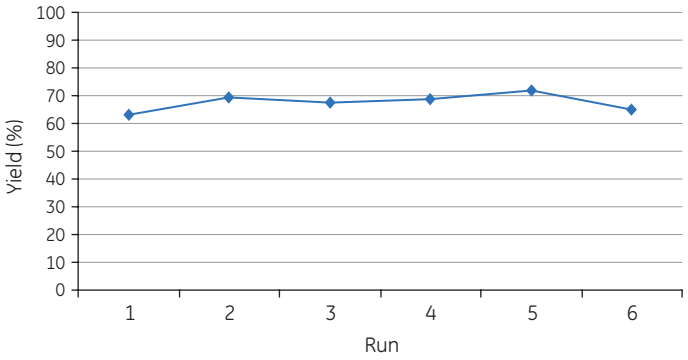


Fig 10. The yield in the eluted pools was retained over the course of the study, which comprised six standard purifications and five intermittent regenerations using 0.5 M NaOH.

Summary

MBPTrap HP 1 ml and 5 ml are convenient prepacked columns for rapid affinity purifications of recombinant proteins tagged with maltose binding protein (MBP). Final purities are high and, as the same column can be used for repeated runs, very reproducible. Binding capacity is high and furthermore, proteins elute in narrow peaks, thus minimizing the need for further concentration steps. The columns are easy to run and scale up on chromatography systems such as ÄKTAdesign. MBPTrap HP columns are compatible with commonly-used aqueous buffers and easily regenerated using sodium hydroxide.

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 No.
MBPTrap HP	5 × 1 ml	28-9187-78
	1 × 5 ml	28-9187-79
	5 × 5 ml	28-9187-80

Related products

Product	Quantity	Code No.
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep™ 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
HiLoad 16/60 Superdex 200 pg	1 × 120 ml	17-1069-01
HiLoad 26/60 Superdex 200 pg	1 × 320 ml	17-1071-01

* Special pack size delivered on specific customer order.

Accessories

Product	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 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 MBPTrap/HiTrap package.

[2] Two, five, or seven stop plugs female included in each MBPTrap/HiTrap packages depending on the product.

[3] One fingertight stop plug is connected to the top of each MBPTrap/HiTrap column at delivery.

Related literature	Code No.
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
Ni Sepharose and IMAC Sepharose, Total solutions for preparation of Histidine-tagged proteins, Selection Guide	28-4070-92
Glutathione Sepharose - Total solutions for preparation of GST-tagged proteins, Selection guide	28-9168-33

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First published Aug. 2007.

Elanders Österwåla 2007

28-9136-33 AA 08/2007