GE Healthcare

Instructions 28-9136-32 AA **HiTrap affinity columns**

MBPTrap HP 1 ml and 5 ml

MBPTrap™ HP is a ready to use HiTrap™ column prepacked with Dextrin Sepharose™ High Performance, a medium for purifying proteins tagged with maltose binding protein (MBP).

Purification is done under physiological conditions and mild elution preserves the activity of the target protein. Thanks to the high specificity of the binding, very high purity is achieved in just one step.

The design of the HiTrap column, together with the robust, high-resolution prepacked medium, provides fast, simple and easy separations in a convenient format. MBPTrap HP columns can be operated with a syringe, a laboratory pump or a liquid chromatography system such as ÄKTAdesign™.

HiTrap

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

Connectorkit

Connectors supplied	Usage	No. supplied
1/16” male/luer female	Connects syringe to top of HiTrap column	1
Tubing connector flangeless/M6 female	Connects tubing (e.g. Peristaltic Pump P-1) to bottom of HiTrap column[1]	1
Tubing connector flangeless/M6 male	Connects tubing (e.g. Peristaltic Pump P-1) to top of HiTrap column[2]	1
Union 1/16” female/ M6 male	Connects original FPLC™ System through bottom of HiTrap column	1
Union M6 female/ 1/16” male	Connects original FPLC System through top of HiTrap column	1
Stop plug female, 1/16”	Seals bottom of HiTrap column	2, 5 or 7

[1] Union 1/16" female/M6 male is also needed.
[2] Union M6 female/1/16" male is also needed.

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1. Description

Medium properties

MBPTrap HP 1 ml and 5 ml columns are prepacked with Dextrin Sepharose High Performance. This robust, high-resolution medium is based on the 34 µm Sepharose High Performance matrix. Due to the small size of the beads, the MBP-tagged protein is eluted in a narrow peak, minimizing the need for further concentration steps.

The MBP-tag is often chosen when higher solubility of the target protein is needed. Purification is performed under physiological conditions and mild elution using maltose preserves the activity of the target protein. These mild conditions may even allow purification of intact protein complexes.

MBPTrap HP tolerates all commonly used aqueous buffers (see Column properties) and is easily regenerated using sodium hydroxide.

Table 1 summarizes the characteristics of prepacked MBPTrap HP columns.

Column properties

HiTrap columns are made of biocompatible polypropylene that does not interact with biomolecules. 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.

Columns can be operated either with a syringe and the supplied luer adapter, a peristaltic pump, or a chromatography system such as ÄKTAdesign.

Note: HiTrap columns cannot be opened or refilled.

Note: To prevent leakage, screw the connector tightly to the adapter.

Table 1. MBPTrap HP characteristics

Matrix	Rigid, highly cross-linked 6% agarose
Average particle size	34 µm
Ligand	Dextrin
Dynamic binding capacity[1]	Approx. 7 mg MBP2*-paramyosin δSal/ml medium (M_r ~70 000, multimer in solution) Approx. 16 mg MBP2*-β galactosidase/ml medium (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	1 and 5 ml/min for 1 and 5 ml columns respectively
Maximum flow rates	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)	2–13
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.

2. General considerations

Recombinant proteins are engineered with MBP-tags to facilitate detection, isolation and purification procedures. In addition, the MBP-tag is often chosen due to its ability to increase the expression level and solubility of the fusion protein. Purification of MBP-tagged protein is done under physiological conditions, which together with mild elution by maltose, preserves the activity of the target protein.

Regeneration is fast and easy to perform using 0.5 M NaOH, which is also used for cleaning the column.

As an alternative, 0.1% SDS can also be used for regeneration. SDS completely eliminates the binding of MBP to dextrin. See also "Regeneration".

3. Operation

Buffer preparation

Use high purity water and chemicals for buffer preparation. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.

Recommended buffers

Binding buffer:

20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, pH 7.4
Optional: 1 mM DTT

Elution buffer:

10 mM maltose in binding buffer

Regeneration buffer:

0.5 M NaOH

or

0.1% SDS

Sample preparation

Adjust the sample to the composition of the binding buffer. Either dilute the sample with binding buffer or buffer exchange using prepacked desalting columns in various formats, see Table 2.

To avoid clogging the column when loading large sample volumes, filter the sample through a 0.45 µm filter or centrifuge it immediately before application

Purification

MBPTrap HP columns can be operated with a syringe, a laboratory pump or a liquid chromatography system such as ÄKTA design.

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the adapter provided) or pump tubing "drop-to-drop" to avoid introducing air into the column.
2. Remove the snap-off end at the column outlet. Wash out the ethanol with at least 5 column volumes (CV) of distilled water or binding buffer.
3. Equilibrate the column with at least 5 CV of binding buffer at 1 ml/min or 5 ml/min for 1 ml and 5 ml columns respectively.
4. Apply the sample using a syringe fitted to the luer adapter or by pumping it onto the column*.
5. Wash with 5 to 10 CV of binding buffer or until no material appears in the effluent.
6. Elute with 5 CV of elution buffer. The eluted fractions can be buffer exchanged using a prepacked desalting column, see Table 2.

* A lower flow rate (0.5 ml/min or 2.5 ml/min for 1 ml and 5 ml columns respectively) can be used during sample application to optimize performance.

Table 2. Prepacked columns for desalting and buffer exchange.

Code No	Column	Loading volume	Elution volume	Application	Comments
17-1408-01	HiTrap Desalting	0.1–1.5 ml	1.3–4.0 ml	For desalting and buffer exchange of protein extracts (M_r>5000).	Prepacked with Sephadex™ G-25 Superfine. Requires a syringe or pump to run.
17-5087-01	HiPrep™ 26/10 Desalting	Up to 15 ml	15–20 ml	For desalting and buffer exchange of protein extracts (M_r>5000).	Prepacked with Sephadex G-25 Fine. Requires a pump to run.
17-0851-01	PD-10 Desalting	1.0-2.5 ml (gravity mode) 1.75-2.5 ml (spin mode)	3.5 ml (gravity mode) Same volume as loaded (spin mode)	Clean-up of biological samples, e.g. proteins and oligosaccharides (M_r> 5000). Sample preparation before downstream analysis such as desalting, buffer exchange and removal of low-molecular weight compounds.	Prepacked with Sephadex G-25. Gravity and spin protocols available
28-9180-04	PD SpinTrap™ G-25	70-130 µl	130 µl		Prepacked with Sephadex G-25. For use with a microcentrifuge
28-9180-06	PD MultiTrap™ G-25	70-130 µl	130 µl		Prepacked with Sephadex G-25. For use with a centrifuge

28-9180-07	PD MiniTrap™ G-25	0.1-0.5 ml (gravity mode) 0.2-0.5 ml (spin mode)	1 ml (gravity mode) Same volume as loaded (spin mode)	Clean-up of biological samples, e.g. proteins and oligosaccharides (M_r> 5000). Sample preparation before downstream analysis such as desalting, buffer exchange and removal of low-molecular weight compounds.	Prepacked with Sephadex G-25. Gravity and spin protocols available
28-9180-08	PD MidiTrap™ G-25	0.5-1.0 ml (gravity mode) 0.75-1.0 ml (spin mode)	1.5 ml (gravity mode) Same volume as loaded (spin mode)		Prepacked with Sephadex G-25. Gravity and spin protocols available
28-9180-10	PD MiniTrap G-10	0.1-0.3 ml	1.0 ml	Clean-up of peptides, small proteins or saccharides larger than M_r 700 before downstream analysis.	Prepacked with Sephadex G-10. Requires gravity to run.
28-9180-11	PD MidiTrap G-10	0.3-0.8 ml	1.5 ml		Prepacked with Sephadex G-10. Requires gravity to run.

Regeneration

1. Regenerate the column with 3 CV distilled water followed by 3 CV 0.5 M NaOH and 3 CV distilled water. Use a flow rate of 0.5 to 1.0 ml/min or 2.5 to 5.0 ml/min for 1 ml and 5 ml columns respectively for NaOH, and 1 ml/min or 5 ml/min respectively for distilled water.
2. Re-equilibrate the column with 5 CV of binding buffer before starting the next purification.

Note: An alternative to the above regeneration is to replace 0.5 M NaOH with 0.1% SDS. Do not regenerate with 0.1% SDS in a cold-room since the SDS may precipitate.

Note: If P-1 pump is used, a maximum flow rate of 1 to 3 ml/min can be run on a MBPTrap HP 1 ml column.

4. Scaling up

Scaling up from 1 ml to 5 ml MBPTrap HP columns is easily performed by increasing sample load and flow rate five-fold.

An alternative method for quick scale-up is to connect two or three MBPTrap HP columns in series (back pressure will increase).

5. Storage

Store MBPTrap HP columns in 20% ethanol at 4 to 8°C.
After storage, equilibrate with binding buffer before use.

6. Troubleshooting

The following tips may be of assistance. If you have further questions about your MBPTrap HP column, please visit *www.gelifesciences.com/hitrap* or contact our technical support or your local GE Healthcare representative.

Increased back pressure:

- Increase the efficiency of the mechanical cell disruption e.g. increase sonication time. (Keep the sample on ice during sonication to avoid frothing and overheating as this may denature the target protein. Over-sonication can also lead to co-purification of host proteins with the target protein).
- Increase dilution of the cell paste before mechanical lysis, or dilute after lysis to reduce viscosity.
- If the lysate is very viscous due to a high concentration of host nucleic acid, continue sonication until the viscosity is reduced, and/or add additional DNAse. Alternatively, draw the lysate through a syringe needle several times.
- Freezing/thawing the unclarified lysate may increase precipitation and aggregation. Sonicating the thawed lysate can prevent increased backpressure problems when loading on the column.
- If the purification has been performed at 4°C, try repeating it at room temperature if possible (sample viscosity is reduced at room temperature).
- Decrease flow rate during sample loading.

Column has clogged:

- Replace the column.
- Optimize sample pretreatment before loading the next sample.

No or weak binding to MBPTrap HP column:

- Protein has precipitated in the column: Decrease the amount of sample, or decrease protein concentration by eluting with a linear gradient instead of step-wise elution.

- Protein found in the flow-through: Buffer/sample composition is not optimal; check the pH and composition of the sample and binding buffer. pH should be above pH 7.
- Column capacity is exceeded: If a MBPTrap HP 1 ml column has been used, change to the larger MBPTrap HP 5 ml. For quick scale-up, connect two or more columns in series by screwing the end of one column into the top of the next. Note, however, that connecting columns in series will increase backpressure.
- Factors in the crude extract interfere with binding: Include glucose in the growth medium to suppress amylase expression.
- MBP-tag is not present: Use protease-deficient *E. coli* expression strains. Add protease inhibitors during cell lysis.
- MBP-tag is not accessible: Fuse the MBP-tag with the other protein terminus. Use another linker.

Contaminating proteins

- Contaminants are short forms of the tagged protein: Use protease-deficient *E. coli* expression strains. Add protease inhibitors after cell lysis. Fuse the MBP-tag with the other protein terminus. Check for the presence of internal translation initiation starts (for C-terminal MBP-tag) or premature termination sites (for N-terminal MBP-tag). Use EDTA in the sample and buffers.
- Contaminants are covalently linked to the recombinant protein via disulfide bonds:
  Add reducing agents to all buffers for cell lysis and purification.
  Note that the yield may decrease.
- Contaminants are non-covalently linked to the recombinant protein: Increase ionic strength in all buffers for cell lysis and purification (up to 1 M NaCl) or add mild detergents (0.1% Triton X-100, 0.1% Tween, 0.1% CHAPS). Be careful since the binding of MBP to dextrin may be affected by the addition of non-ionic detergents.

Unwanted air bubble formation

- Unclarified lysates may increase air bubble formation during purification. Attaching a flow restrictor in the chromatography system can prevent this. If a flow restrictor is attached, it is important to change the pressure limit to 0.5 MPa (5 bar) on the ÄKTAdesign system (the column and flow restrictor give a pressure of 0.3 MPa and 0.2 MPa, respectively).
- Air bubbles may form due to decreased air solubility when columns stored at 4 to 8°C are used immediately at room temperature. Let the columns adapt to room temperature for some minutes before using them.

7. Further information

Refer to New England Biolabs for expression, detection and/or assays for MBP-tagged proteins. For further information, visit www.gelifesciences.com/hitrap or www.gelifesciences.com/protein-purification or contact your local GE Healthcare representative.

8. Ordering Information

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

Related products	No. Supplied	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
PD-10 Desalting Columns	30	17-0851-01
PD SpinTrap G-25	50	28-9180-04
PD MultiTrap G-25	4 × 96-well plates	28-9180-06
PD MiniTrap G-25	50	28-9180-07
PD MidiTrap G-25	50	28-9180-08
PD MiniTrap G-10	50	28-9180-10
PD MidiTrap G-10	50	28-9180-11

* Special pack size delivered on specific customer order.

Accessories	No. Supplied	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
Finger-tight 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 each HiTrap packages depending on the product.
[3] One finger-tight stop plug is connected to the top of each 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 Selection 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

www.gelifesciences.com/hitrap
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