Instructions 28-9549-01 AB

HiTrap™ Con A 4B, 1 ml and 5 ml

HiTrap Con A 4B is a ready to use column prepacked with Con A Sepharose™ 4B, a medium for separation and purification of glycoproteins, polysaccharides and glycolipids.

The design of the HiTrap column, together with the prepacked medium provides simple and easy separations in a convenient format. HiTrap Con A 4B columns can be operated with a syringe, a peristaltic pump or a liquid chromatography system such as ÄKTA™.

Table of contents

2. Product description 3
2. General considerations 6
3. Operation 7
4. Scaling up 10
5. Adjusting pressure limits in chromatography system software 11
6. Storage 12
7. Ordering Information 13

Please read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

1 Product description

HiTrap column characteristics

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

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

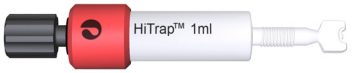

Fig 1. HiTrap, 1 ml column.

Fig 2. HiTrap, 5 ml column.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 ml	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 x 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

Medium properties

Con A Sepharose is Concanavalin A coupled to Sepharose 4B by the cyanogen bromide method.

Concanavalin A (Con A) is a tetrameric metalloprotein isolated from *Canavalia ensiformis* (jack bean). Con A binds molecules containing α-D-mannopyranosyl, α-D-glucopyranosyl and sterically related residues. The binding sugar requires the presence of C-3, C-4 and C-5 hydroxyl groups for reaction with Con A. Con A coupled to Sepharose is routinely used for separation and purification of glycoproteins, polysaccharides and glycolipids.

Other application areas where Con A Sepharose 4B has been used are purification of enzyme-antibody conjugates, purification of IgM, isolation of cell surface glycoproteins from detergent-solubilized membranes, separation of membrane vesicles, and the study of changes in composition of carbohydrate-containing substances.

The characteristics of HiTrap Con A 4B column are summarized in Table 2.

Table 2. HiTrap Con A 4B characteristics

Matrix	4% agarose
Average particle size	90 µm
Ligand	Concanavalin A
Ligand concentration	10 to 15 mg Con A/ml medium
Binding capacity	20 to 45 mg porcine thyroglobulin/ml medium
Recommended flow rates	0.1 to 1 ml/min (1 ml) 0.5 to 5 ml/min (5 ml)

Maximum flow rates	4 ml/min (1 ml) 20 ml/min (5 ml)
Chemical stability	Stable to all commonly used aqueous buffers. Chelating agents such as EDTA, 8 M urea or solutions having a pH below 3 should be avoided as these conditions results in removal of manganese from the lectin with loss of activity as a result.
pH stability	4 to 9
Storage	4ºC to 8ºC in 0.1 M acetate buffer pH 6 containing 1 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 1 mM $MgCl_2$. Use 20% ethanol as preservative.

2 General considerations

Binding

The most important parameter affecting the binding of glycoproteins to the immobilized lectin is the flow rate. It is important to keep the flow rate as low as 0.1 to 1 ml/min (1 ml column) or 0.5 to 5 ml/min (5 ml column) during sample application for maximum binding capacity. This is especially important for samples containing detergents as the binding activity decreases in the presence of detergents. An alternative to keeping the flow rate low is to apply 1 ml or 5 ml sample (depending on the column size) at the time and let it bind for a couple of minutes. Repeat this procedure until all the sample is applied to the column.

Binding of glycoproteins and carbohydrate containing proteins occurs at neutral pH. The binding of substances to Con A Sepharose 4B requires the presence of both Mn^{2+} and Ca^{2+}. The protein-metal ion complex remains active and is stable at neutral pH even in the absence of the free metal ions. However to preserve the binding activity of the Con A molecule below pH 5, excess Mn^{2+} and Ca^{2+} (1 mM) must be present. This will ensure an active Con A-metal complex.

Elution

Elution of bound substances can be achieved using an increasing gradient (linear or step) of methyl-α-D-mannopyranoside (methyl-α-D-mannoside) or methyl-α-D-glucopyranoside (methyl-α-D-glucoside). These sugars act as strong eluents. Many substances elute at 0.1 to 0.2 M but higher concentrations may be required for more tightly bound substances. Glucose and mannose may also be used but are weaker eluents. The recovery of glycoproteins can sometimes be improved by pausing the flow for a couple of minutes during elution. Tightly bound substances may also be eluted by lowering the pH, but not below pH 4.

Borate is known to form complexes with cis-diols on sugar residues and thus act as an competing eluent. For elution with borate, use a 0.1 M borate buffer, pH 6.5. Recovery on HiTrap Con A 4B is decreased in the presence of detergents.

3 Operation

Recommended buffers

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

Binding buffer:

20 mM Tris-HCl, 0.5 M NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.4.

Elution buffer:

0.1 to 0.5 M methyl-α-D-glucopyranoside (methyl-α-D-glucoside) or methyl-α-D-mannopyranoside (methyl-α-D-mannoside), 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4

Note: *Before reuse the column has to be regenerated by washing with 10 CV of 20 mM Tris-HCl, 0.5 M NaCl, pH 8.5 followed by re-equilibration with binding buffer. The reuse of HiTrap Con A 4B depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.*

Sample preparation

Adjust the sample to the composition of the binding buffer. Either dilute the sample with binding buffer or buffer exchange using HiTrap Desalting, HiPrep™ 26/10 Desalting or Desalting PD-10 column, see Table 2.

Filter the sample through a 0.45 µm filter or centrifuge it immediately before application.

Purification

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 storage solution with 5 to 10 column volumes (CV) of distilled water or binding buffer.

3 Equilibrate the column with 5 to 10 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. For the best results use a low flow rate, 0.1 to 0.5 ml/min or 0.5 to 2.5 ml/min for 1 ml and 5 ml columns respectively.

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 HiTrap Desalting, HiPrep 26/10 Desalting or Desalting PD-10 column, see Table 3.

Note: *Recovery can sometimes be improved by pausing the flow for some minutes during elution.*

Table 3. Prepacked columns for desalting and buffer exchange

Code No.	Column	Loading volume	Elution volume
17-1408-01	HiTrap Desalting	0.1 to 1.5 ml	1.3 to 4.0 ml
17-5087-01	HiPrep 26/10 Desalting	Up to 15 ml	15 to 20 ml
17-0851-01	PD-10 Desalting	1.0 to 2.5 ml (gravity mode) 1.75 to 2.5 ml (spin mode)	3.5 ml (gravity mode) Same volume as loaded (spin mode)
28-9180-04	PD SpinTrap™ G-25	70 to 130 µl	130 µl
28-9180-06	PD MultiTrap™ G-25	70 to 130 µl	130 µl
28-9180-07	PD MiniTrap™ G-25	0.1 to 0.5 ml (gravity mode) 0.2 to 0.5 ml (spin mode)	1 ml (gravity mode) Same volume as loaded (spin mode)
28-9180-08	PD MidiTrap™ G-25	0.5 to 1.0 ml (gravity mode) 0.75 to 1.0 ml (spin mode)	1.5 ml (gravity mode) Same volume as loaded (spin mode)
28-9180-10	PD MiniTrap G-10	0.1 to 0.3 ml	1.0 ml
28-9180-11	PD MidiTrap G-10	0.3 to 0.8 ml	1.5 ml

Application	Comments
For desalting and buffer exchange of protein extracts (M_r>5000).	Prepacked with Sephadex™ G-25 Superfine. Requires a syringe or pump to run.
For desalting and buffer exchange of protein extracts (M_r>5000).	Prepacked with Sephadex G-25 Fine. Requires a pump to run.
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.
	Prepacked with Sephadex G-25. For use with a microcentrifuge.
	Prepacked with Sephadex G-25. For use with a centrifuge.
	Prepacked with Sephadex G-25. Gravity and spin protocols available.
	Prepacked with Sephadex G-25. Gravity and spin protocols available.
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.
	Prepacked with Sephadex G-10. Requires gravity to run.

4 Scaling up

Scaling up from 1 ml to 5 ml HiTrap Con A 4B 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 HiTrap Con A 4B columns in series (back pressure will increase).

5 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 3. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

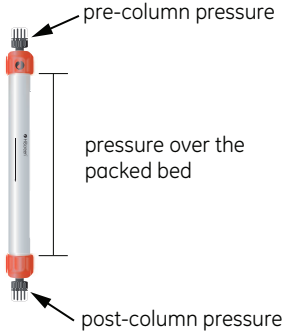

Fig 3. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

1. Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2. Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3. Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

   The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

6 Storage

Store columns at 4°C to 8°C in 0.1 M acetate buffer pH 6 containing 1 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 1 mM $MgCl_2$. Use 20% ethanol as preservative. After storage, equilibrate with binding buffer before use.

7 Ordering Information

Product	No. Supplied	Code No.
HiTrap Con A 4B	5 x 1 ml	28-9520-85
HiTrap Con A 4B	5 x 5 ml	28-9520-96

Related products	No. Supplied	Code No.
Con A Sepharose 4B	5 ml	17-0440-03
Con A Sepharose 4B	100 ml	17-0440-01
Lentil Lectin Sepharose 4B	25 ml	17-0444-01
HiTrap Desalting	1 x 5 ml 5 x 5 ml 100 x 5 ml*	29-0486-84 17-1408-01 11-0003-29
HiPrep 26/20 Desalting	1 x 53 ml 4 x 53 ml	17-5087-01 17-5087-02
PD-10 Desalting Columns	30	17-0851-01

* Special pack size delivered on specific customer order.

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column)*	5	18-3858-01

Accessories	Quantity	Code No.
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" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography, Columns and Media Selection Guide	18-1121-86
Prepacked chromatography columns for ÄKTAdesign systems, Selection Guide	28-9317-78

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