cytiva

HiTrap Con A 4B, 1 mL and 5 mL

Prepacked columns

Instructions for Use

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

The design of the HiTrap column, together with the prepacked resin, 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™.

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Important

Read these instructions carefully before using the products.

Intended use

The products 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 products in a safe way, refer to the *Safety Data Sheets*.

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. The table below lists the characteristics of HiTrap columns.

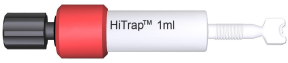

Fig 1. HiTrap, 1 mL column

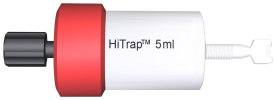

Fig 2. HiTrap, 5 mL column

Note: *HiTrap columns must not 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 × 2.5 cm
Column hardware pressure limit	0.5 MPa (5 bar)	0.5 MPa (5 bar)

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

Supplied connector kit

Table 2. Connector kit supplied 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

Resin 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 the following table.

Table 3. Characteristics of HiTrap Con A 4B

Matrix	4% agarose
Particle size, d_{50V} [1]	~ 90 µm
Ligand	Concanavalin A
Ligand concentration	10 to 15 mg Con A/mL resin
Binding capacity[2]	20 to 45 mg porcine thyroglobulin/mL resin
Recommended flow rates	0.1 to 1 mL/min (1 mL) 0.5 to 5 mL/min (5 mL)
Maximum flow rates	1 mL/min (1 mL) 5 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	2°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.

[1] Median particle size of the cumulative volume distribution.

[2] The dynamic binding capacity can be optimized for process development. Increased residence time gives higher dynamic binding capacity.

2 General considerations

Wash out unbound Con A

To avoid contamination of leaked monomeric Con A (M_r = 25 000) in eluted fractions a 10 column volume (CV) wash procedure should be performed prior purification with binding buffer. If contaminations still are present, prolong the wash step until all unbound monomeric Con A has been removed.

Binding

The most important parameter affecting the binding of glycoproteins to the immobilized lectin is the flow rate. For maximum binding capacity it is important to keep the flow rate low during sample application. 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 might 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 can 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

Step	Action
1	Adjust the sample to the composition of the binding buffer. Either dilute the sample with binding buffer or perform buffer exchange using HiTrap Desalting, HiPrep™ 26/10 Desalting, or Desalting PD-10 column, see *Table 4, on page 9*.
2	Filter the sample through a 0.45 µm filter or centrifuge it immediately before application.

Purification

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

Step	Action
1	Fill the syringe or pump tubing with binding buffer.
2	Remove the stopper and connect the column to the syringe or pump tubing—"drop-to-drop"—to avoid introducing air into the column. Use the provided luer adapter for the syringe.

Step	Action
3	Remove the snap-off end at the column outlet. Wash out the storage solution with 5 to 10 CV of distilled water or binding buffer.
4	Equilibrate the column with at least 10 CV binding buffer at 1 mL/min or 5 mL/min for 1 mL and 5 mL columns respectively to make sure that unbound Con A has been removed.
5	Apply the sample using a syringe fitted to the luer adapter or by pumping it onto the column. Use low flow rates: 0.1 to 0.5 mL/min, or 0.5 to 2.5 mL/min for 1 mL and 5 mL columns respectively.
6	Wash with 5 to 10 CV binding buffer or until no material appears in the effluent.
7	Elute with 5 CV elution buffer. The eluted fractions can be buffer exchanged using a HiTrap Desalting, HiPrep 26/10 Desalting, or Desalting PD-10 column, see *Table 4, on page 9*.

Prepacked columns for desalting and buffer exchange

The prepacked columns described in the Table below are used for desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).

Table 4. Prepacked columns for desalting and buffer exchange

Column	Loading volume	Elution volume
HiTrap Desalting[1]	0.25 to 1.5 mL	1.0 to 2.0 mL
HiPrep 26/10 Desalting[2]	2.5 to 15 mL	7.5 to 20 mL

Column	Loading volume	Elution volume
PD-10 Desalting[3]	1.0 to 2.5 mL[4]	3.5 mL[4]
	1.75 to 2.5 mL[5]	Up to 2.5 mL[5]
PD SpinTrap™ G-25	70 to 130 µL	130 µL
PD MultiTrap™ G-25	70 to 130 µL	130 µL
PD MiniTrap™ G-25	0.1 to 0.25 mL[4]	1.0 mL[4]
	0.2 to 0.5 mL[5]	Up to 0.5 mL[5]
PD MidiTrap™ G-25	0.5 to 1 mL[4]	1.5 mL[4]
	0.75 to 1 mL[5]	Up to 1 mL[5]
PD MiniTrap G-10	0.1 to 0.3 mL	1.0 mL
PD MidiTrap G-10	0.3 to 0.8 mL	1.0 mL

1 Prepacked with Sephadex™ G-25 Superfine and requires a syringe or pump to run.

2 Prepacked with Sephadex G-25 Fine and requires a pump or a chromatography system to run.

3 Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

4 Volumes with gravity elution (gravity mode).

5 Volumes with centrifugation (spin mode).

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.

Note: *The back pressure will increase when the columns are connected in series. This can easily be addressed by lowering the flow rate.*

5 Adjusting pressure limits

The pressure generated by the flow through a column affects the packed bed and the column hardware, refer to the figure below. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- High viscosity for buffers or sample
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit can damage the column, refer to* *Table 3, on page 5**.*

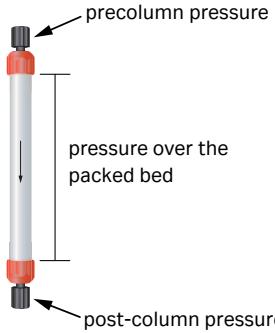

Fig 3. Precolumn and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (precolumn pressure and pressure over the packed bed, Δp). The precolumn pressure limit is the column hardware pressure limit refer to *Table 3, on page 5*.

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

Chromatography systems with pressure sensor in the pump

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

Step	Action
1	Replace the column with a piece of tubing.
	a. Run the pump at the maximum intended flow rate.
	b. Record 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: *There will be a drip from the column valve.*
	a. Record the pressure as P2.

Step	Action
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit, see *Table 1, on page 3*.
	a. Replace the pressure limit in the software with the calculated value.

The actual pressure over the packed bed (Δp) during the run is equal to the actual measured pressure which is the total system pressure (P1).

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

6 Storage

Store columns at 2°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

Table 5. Products

Product	Quantity	Product code
HiTrap Con A 4B	5 × 1 mL	28952085
	5 × 5 mL	28952096

Table 6. Related products

Product	Quantity	Product code
Con A Sepharose 4B	5 mL	17044003

Product	Quantity	Product code
	100 mL	17044001
Lentil Lectin Sepharose 4B	25 mL	17044401
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702
PD-10 Desalting Columns	30	17085101

Table 7. Accessories

Product	Quantity	Product code
1/16″ male/luer female	2	18111251
(For connection of syringe to top of HiTrap column)		
Fingertight connector 1/16″ male, narrow	8	28401081
Stop plug, female 1/16″	5	11000464
(For sealing bottom of HiTrap column)		
Fingertight stop plug 1/16″	5	11000355
PD MiniTrap G-25	50	28918007
PD MidiTrap G-25	50	28918008

Table 8. Related literature

Product	Product code
Affinity Chromatography – Vol. 3: Specific Groups of Biomolecules	18102229
Affinity Chromatography, Columns and Media Selection Guide	18112186
Prepacked chromatography columns for ÄKTA systems, Selection guide	28931778

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