Instructions 71-5004-39 AK

HiTrap™ Streptavidin HP, 1 ml

HiTrap Streptavidin HP is a prepacked, ready to use, column for preparative affinity chromatography. The special design of the column, together with the matrix, provides fast, simple and easy separations in a convenient format.

The column can be operated with a syringe, a peristaltic pump or a liquid chromatography system such as ÄKTA™.

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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.

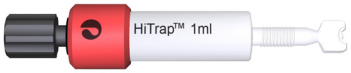

Fig 1. HiTrap, 1 ml column.

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
Column dimensions	0.7 × 2.5 cm
Column hardware pressure limit	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

Purified Streptavidin isolated from Streptomyces avidinii is immobilized on Sepharose™ High Performance. The immobilized streptavidin binds biotin and biotinylated substances and can be used for affinity chromatography applications. For examples of use, see refs. 1–11. The interaction between streptavidin and biotin is very strong and requires denaturing conditions for elution, which may destroy both the ligand and the sample. Alternatively, it can be used in the purification of antigens, where biotinylated antibodies are incubated with antigen. The biotinylated antibody-antigen complex binds to HiTrap Streptavidin HP from which the antigen can be eluted (refs. 9–10). Another example is to utilize the interaction between 2-iminobiotin and streptavidin, eluting the bound substances at pH 4 (ref. 11).

The characteristics of the product are summarized below.

Table 2. HiTrap Streptavidin HP characteristics.

Ligand	Streptavidin
Binding capacity	Biotin: > 300 nmol/ml medium Biotinylated bovine serum albumin: 6 mg/ml medium
Mean particle size	34 µm
Bead structure	Highly cross-linked spherical agarose
Maximum flow rate	4 ml/min
Recommended flow rate	0.1 to 1 ml/min
pH stability	
Short term	2 to 10.5
Long term	4 to 9
Temperature stability	
Regular use	4°C to room temperature
Storage	4°C to 8°C
Storage buffer	20% ethanol

Note: *Streptavidin Sepharose High Performance is also available as lab packs in 5 ml packages.*

2 Binding of biotin or biotinylated substances

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use

Binding	20 mM sodium phosphate, 0.15 M NaCl, pH 7.5
Elution	8 M guanidine-HCl, pH 1.5.

Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done either by diluting the sample with binding buffer or by buffer exchange using HiTrap Desalting, HiPrep™ 26/10 Desalting or PD-10 Desalting columns. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column.

Purification

The recommended flow rate for HiTrap Streptavidin HP is 1 ml/min, unless otherwise stipulated.

Note: *1 ml/min corresponds to approximately 30 drops/min when the column is operated with a syringe.*

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper. To avoid introducing air into the column, connect the column "drop to drop" to either the syringe (via the luer connector) or to the pump tubing.
2. Remove the snap-off end at the column outlet.
3. Equilibrate the column with 10 column volumes of binding buffer.
4. Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column. For best results use a low flow rate, 0.1–0.5 ml/min, during sample application.

5 Wash with at least 10 column volumes of binding buffer or until no material appears in the effluent.

6 Elute with 10–20 column volumes of elution buffer. To protect the sample, adjust the pH of the eluate with buffer exchange, for example on a HiTrap Desalting or a PD-10 Desalting column.

Note: *The harsh conditions required to break the streptavidinbiotin bond may affect both the sample and the ligand. HiTrap Streptavidin HP columns can not be re-used after elution under these conditions.*

Note: *If a P1-pump is used a max flow rate of 1–3 ml/min can be be run on a HiTrap 1 ml column packed with Sepharose High Performance media.*

3 Purification of iminobiotinylated substances

An alternative to labelling the sample with biotin is to use 2-iminobiotin which binds to streptavidin above pH 9.5 and can be eluted at pH 4.

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

Binding	50 mM ammonium carbonate, 0.5 M NaCl, pH 10.0
Elution	50 mM ammonium acetate, 0.5 M NaCl, pH 4.0

Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done either by diluting the sample with binding buffer or by buffer exchange using HiTrap Desalting or PD-10 columns. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column.

Purification

The recommended flow rate for HiTrap Streptavidin HP is 1 ml/min, unless otherwise is stipulated.

Note: *1 ml/min corresponds to approximately 30 drops/min when the column is operated with a syringe.*

1 Fill the syringe or pump tubing with binding buffer. Remove the stopper. To avoid introducing air into the column, connect the column "drop to drop" to either the syringe (via the luer connector) or to the pump tubing.

2 Remove the snap-off end at the column outlet.

3 Equilibrate the column with at least 10 column volumes of binding buffer.

4 Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column. For best results use a low flow rate, 0.1–0.2 ml/min, during sample application.

5 Wash with at least 10 column volumes of binding buffer or until no material appears in the effluent.

6 Elute with 5–10 column volumes of elution buffer. To protect the sample, adjust the pH of the eluate with buffer exchange, for example on a HiTrap Desalting or a PD-10 Desalting column.

Note: *The re-use of HiTrap Streptavidin HP depends on the nature of the sample and should only be performed with identical samples to avoid cross-contamination.*

Note: *If a P1-pump is used a max flow rate of 1–3 ml/min can be be run on a HiTrap 1 ml column packed with Sepharose High Performance media.*

4 Binding of antibodies

Biotinylated antibody-antigen complexes bind to HiTrap Streptavidin HP from which the antigen can be purified. The following method was adapted for HiTrap Streptavidin HP from work published by Gretch et al (ref. 10).

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

Solubilization buffer: 20 mM sodium phosphate, 150 mM NaCl, pH 7.5 with 0.1% SDS, 1.0% NP-40, 0.5% sodium deoxycholate, 0.02% NaN_3, 100 µg/ml PMSF

Elution buffer: 0.1 M glycine-HCl, pH 2.2

Sample preparation

All steps should be performed at 4°C.

1. Solubilize the antigen with an appropriate amount of solubilization buffer, clear the lysate by centrifuging at 12 000 × g for 15 min.
2. Add the biotinylated antibody.
3. Adjust the volume to 1 ml.
4. Incubate with end-over-end mixing, for at least 1 hour or overnight.

Purification

The recommended flow rate for HiTrap Streptavidin HP is 1 ml/min, unless other flow rate is stipulated.

Note: *1 ml/min corresponds to approximately 30 drops/s when the column is operated with a syringe.*

All steps should be performed at 4°C.

1. Fill the syringe or pump tubing with solubilization buffer. Remove the stopper. To avoid introducing air into the column, connect the column "drop to drop" to either the syringe (via the luer connector) or to the pump tubing.
2. Remove the snap-off end at the column outlet.
3. Equilibrate the column with 10 column volumes solubilization buffer.
4. Apply antibody-antigen solution to the column at a low flow rate 0.2 ml/min. If the sample volume is less than 1 ml, apply all of the sample at once, then stop the flow to let it bind for a few minutes.

5 Wash out unbound sample with at least 10 column volumes of solubilization buffer or until no material is found in the flow through.

6 Elute with 5–10 column volumes of elution buffer. To protect the sample, immediately adjust the pH of the eluate to neutral by addition of 100–200 µl of 1.0 M Tris-HCl, pH 9, per ml fraction, or by buffer exchange on a HiTrap Desalting or a PD-10 Desalting column.

Note: *The reuse of HiTrap Streptavidin HP depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.*

Note: *If a P1-pump is used a max flow rate of 1–3 ml/min can be run on a HiTrap 1 ml column packed with Sepharose High Performance media.*

5 Scaling up

For quick scale-up of purification, two or three HiTrap columns can be connected in series (back pressure will increase). For further scale-up bulk media is available, *see* Ordering information.

6 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 2. 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.*

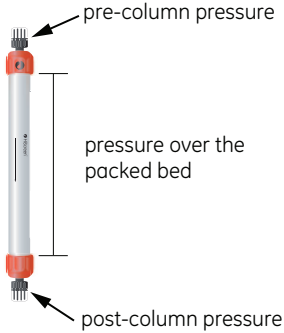

Fig 2. Pre-column and post-column measurements.

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.*

7 Storage

Store the column at 4°C to 8°C in 20% ethanol.

8 Ordering information

Product	No. Supplied	Code No.
HiTrap Streptavidin HP	5 × 1 ml	17-5112-01

Related Products	No. Supplied	Code No.
Streptavidine Sepharose High	5 ml	17-5113-01
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
PD-10 Desalting Column	30	17-0851-01

Accessories	No. Supplied	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
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

9 References

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