cytiva

StrepTrap HP, 1 ml and 5 ml

Instructions for Use

StrepTrap™ HP is a ready to use HiTrap™ column prepacked with StrepTactin Sepharose™ High Performance, a medium for purifying *Strep*-tag™ II proteins.

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

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Important

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

StrepTrap HP 1 ml and 5 ml columns are prepacked with StrepTactin 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 *Strep*-tag II protein is eluted in a narrow peak minimizing the need for further concentration steps.

The immobilized *Strep-Tactin*™ ligand is a specially-engineered streptavidin. The binding affinity of the *Strep*-tag II to *Strep-Tactin* is nearly 100-fold higher than to streptavidin. Purification is performed under physiological conditions and mild elution using desthiobiotin preserves the activity of the target protein. The mild conditions even allow purification of intact protein complexes.

StrepTactin Sepharose High Performance is compatible with a wide range of additives (see Table 3) and is easily regenerated using sodium hydroxide.

Table 2 summarizes the characteristics of prepacked StrepTrap HP columns.

Table 2. StrepTactin Sepharose High Performance characteristics.

Matrix	Rigid highly cross-linked 6% agarose
Average particle size	34 µm
Ligand	*Strep-Tactin*
Ligand concentration	Approx. 5 mg/ml medium
Dynamic binding capacity[1]	Approx. 6 mg *Strep*-tag II protein/ml medium
Recommended flow rates	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
Chemical stability	Stable in all commonly used aqueous buffers reducing agents, and detergents (see Table 3)
pH working range	> pH 7.0
Storage	2°C to 8°C in 20% ethanol

[1] Binding capacity is protein dependent. Dynamic binding capacity (DBC) was tested here with GAPDH-*Strep*-tag II, M_r 37 400.

[2] H_2O at room temperature.

Table 3. Compatibility of StrepTactin Sepharose High Performance with different additives[1]

Reagent	Concentration
DTT	50 mM
β-mercaptoethanol	50 mM
Non-ionic detergents	
C8E4, Octyltetraoxyethylene	max. 0.88%
C10E5, Decylpentaoxyethylene	0.12%
C10E6	0.03%
C12E8	0.005%
C12E9, Dodecyl nonaoxyethylene (Thesit)	0.023%
Decyl-β-D-maltoside	0.35%
N-dodecyl-β-D-maltoside	0.007%
N-nonyl-β-D-glucopyranoside	0.2%
N-octyl-β-D-glucopyranoside	2.34%
Triton™ X-100	2%
Tween™ 20	2%
Ionic detergents	
N-lauryl-sarcosine	2%
8-HESO;N-octyl-2-hydroxy-ethylsulfoxide	1.32%
SDS, Sodium-N-dodecyl sulphate	0.1%
Zwitterionic detergents	
CHAPS	0.1%
DDAO, N-decyl-N,N-dimethylamine-N-oxide	0.034%
LDAO, N-dodecyl-N,N-dimethylamine-N-oxide	0.13%
Others	
Ammonium sulphate, $(NH_4)_2SO_4$	2 M
$CaCl_2$	max. 1 M
EDTA	50 mM
Guanidine	max. 1 M
Glycerol	max. 25%[2]
Imidazole	500 mM[3]
Reagent	**Concentration**

Others - *continued*	
$MgCl_2$	1 M
Urea	max. 1 M
NaCl	5 M

Note: These reagents have been successfully tested for purifying GAPDH-*Strep*-tag II, for example, with concentrations up to those listed. Higher concentrations may, however, be possible for reagents not marked with "max." Since binding depends on the sterical accessibility of the *Strep*-tag II in the context of the particular protein, the possible concentration may deviate from the given value for other proteins.

[1] Data kindly provided by IBA GmbH, Germany, the manufacturer and IP owner of the *Strep-Tactin* ligand.

[2] Yield may decrease.

[3] 500 mM imidazole in sample tested by Cytiva.

2 General considerations

Strep-tag II is a small tag of only eight amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) and and a molecular weight of 1000. The small size of the tag makes it very useful as it will generally not interfere with structural and functional studies. Thus, it is not always necessary to cleave it off.

To optimize binding properties, streptavidin has been specially engineered to *Strep-Tactin*. In addition, the optimal binding partner has been found in combination with the *Strep*-tag II.

Purification is done under physiological conditions, which together with mild elution by desthiobiotin (a specific competitor that displaces the *Strep*-tag II protein) preserves the activity of the target protein.

Regeneration of the medium is recommended before performing the next purification run on the same column. This is fast and easy to perform using 0.5 M NaOH, which is also used for cleaning the column.

As an alternative, HABA, (2-[4'-hydroxy-benzeneazo] benzoic acid) can also be used for regeneration. (HABA in excess displaces the bound desthiobiotin). 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:

100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8
or
PBS: 20 mM sodium phosphate, 280 mM NaCl, 6 mM potassium chloride, pH 7.4.

Elution buffer:

2.5 mM desthiobiotin in binding buffer.

Regeneration buffer:

0.5 M NaOH
or
1 mM HABA (2-[4'-hydroxy-benzeneazo] benzoic acid) in binding buffer.

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 4.

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

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 5 CV 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 binding buffer or until no material appears in the effluent.

6 Elute with 6 CV elution buffer. The eluted fractions can be buffer exchanged using a HiTrap Desalting, HiPrep 26/10 Desalting or Desalting PD-10 column.

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 with NaOH, and 1 ml/min or 5 ml/min respectively for distilled water.

2 Re-equilibrate the column with 5 CV binding buffer before starting the next purification.

Note: *An alternative to the above regeneration/re-equilibration is 15 CV 1 mM HABA (2-[4'-hydroxy-benzeneazo] benzoic acid) in binding buffer followed by 30 CV binding buffer. Use a flow rate of 2 ml/min or 10 ml/min for 1 ml and 5 ml columns respectively. The displacement is detected by the change in color of the medium in the column from yellow to red. This color change is due to the accumulation of HABA/Strep-Tactin complexes. The HABA is washed away with the binding buffer.*

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

4 Scaling up

Scaling up from 1 ml to 5 ml StrepTrap 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 StrepTrap HP 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.*

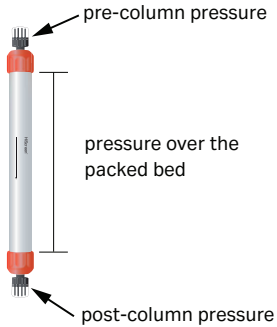

Fig 3. Pre-column and post-column measurements.

ÄKTA avant

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 StrepTrap HP columns in 20% ethanol at 2°C to 8°C.

After storage, equilibrate with binding buffer before use.

Table 4. Prepacked columns for desalting and buffer exchange.

Code No	Column	Loading volume	Elution volume
17-1408-01	HiTrap Desalting	0.1–1.5 ml	1.3–4.0 ml
17-5087-01	HiPrep 26/10 Desalting	Up to 15 ml	15–20 ml
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)
28-9180-04	PD SpinTrap™ G-25	70-130 µl	130 µl
28-9180-06	PD MultiTrap™ G-25	70-130 µl	130 µl
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)
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)
28-9180-10	PD MiniTrap G-10	0.1-0.3 ml	1.0 ml
28-9180-11	PD MidiTrap G-10	0.3-0.8 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.

7 Troubleshooting

The following tips may be of assistance. If you have further questions about your StrepTrap HP column, please visit *cytiva.com/hitrap* or contact our technical support or your local Cytiva 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 StepTrap 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. Try detergents or change the NaCl concentration.
- Protein found in the flowthrough: 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 StrepTrap HP 1 ml column has been used, change to the larger StrepTrap 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.
- *Strep*-tag II is not present: Use protease-deficient *E. coli* expression strains. Add protease inhibitors during cell lysis.
- *Strep*-tag II is not accessible: Fuse *Strep*-tag II with the other protein terminus: Use another linker.
- The ligand is blocked by biotinylated proteins from the extract: Add avidin (Biotin Blocking Buffer) if biotin-containing extracts are to be purified. The biotin content of the soluble part of the total *E. coli* cell lysate is about 1 nmol per liter culture (OD 550 = 1.0). Add 2 to 3 nmol of avidin monomer per nmol of biotin.

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 *Strep*-tag II with the other protein terminus. Check for the presence of internal translation initiation starts (for C-terminal *Strep*-tag II) or premature termination sites (for N-terminal *Strep*-tag II). 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.
- 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% Tween, 0.1% CHAPS).

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 ÄKTA system (the column and flow restrictor give a pressure of 0.3 MPa and 0.2 MPa, respectively).
- Air bubble formation may occur due to decreased air solubility when columns stored at 4°C to 8°C are immediately used at room temperature.
  Let the columns adapt to room temperature for some minutes before start using them.

8 Further information

Refer to IBA GmbH, Germany, www.iba-go.com, for expression, detection and/or assays for *Strep*-tag II proteins. For further information, visit
cytiva.com/hitrap or cytiva.com/protein-purification or contact your local Cytiva representative.

9 Ordering Information

Product	No. Supplied	Code No.
StrepTrap HP	1 × 1 ml	29-0486-53
	5 × 1 ml	28-9075-46
	1 × 5 ml	28-9075-47
	5 × 5 ml	28-9075-48

Related products	No. Supplied	Code No.
HiTrap Desalting	1 × 5 ml	29-0486-84
	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

* 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
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.
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
Prepacked chromatography columns for ÄKTAdesign systems, Selection guide	28-9317-78

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