

StrepTactin Sepharose High Performance

Introduction

StrepTactin™ Sepharose™ High Performance is a chromatography medium for purifying Strep(II)-tagged 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 StrepTactin ligand bound to the matrix is a specially engineered streptavidin ligand. The binding affinity of the Strep(II)-tag to the immobilized ligand is nearly 100-fold higher than to streptavidin, making StrepTactin Sepharose High Performance ideal for purifying Strep(II)-tagged proteins.

StrepTactin Sepharose High Performance is available in 10 and 50 ml lab packs and prepacked in 1 and 5 ml StrepTrap™ HP columns.



Intended use

StrepTactin Sepharose High Performance is intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

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1 Description

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(III)-tagged protein is eluted in a narrow peak minimizing the need for further concentration steps.

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.

Table 1 summarizes the characteristics of StrepTactin Sepharose High Performance chromatography medium.

Table 1. StrepTactin Sepharose High Performance chromatography medium characteristics.

Matrix	Rigid, highly cross-linked agarose
Average particle size	34 μm
Ligand	StrepTactin
Ligand concentration	Approx. 5 mg/ml medium
Dynamic binding capacity¹	Approx. 6 mg Strep(III)-tagged protein/ml medium
Max. linear flow rate²	300 cm/h
Recommended linear flow rate²	\leq 150 cm/h
Maximum back pressure²	0.3 MPa, 3 bar
Chemical stability	Stable in all commonly used buffers, 0.5 M NaOH (regeneration and cleaning), reducing agents and detergents (see Table 2)
pH, working range	> pH 7.0
Storage	4 to 8°C in 20% ethanol

¹ Dynamic binding capacity (DBC) is defined as mg protein applied per ml medium at the point where the concentration of protein in the column effluent reaches a value of 10% of the concentration in the sample. DBC was tested here with GAPDH-Strep(III), M_r 37 400. Binding capacity is protein to protein dependent.

² H₂O at room temperature.

StrepTactin Sepharose High Performance is compatible with a wide range of additives (see Table 2) and is easily regenerated using 0.5 M sodium hydroxide.

Table 2. Compatibility of StrepTactin Sepharose High Performance with different additives¹.

Additive	Concentration
Reduction agents	
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) ₂ SO ₄	2 M
CaCl ₂	max. 1 M
EDTA	50 mM
Guanidine	max. 1 M
Glycerol	max. 25% ³
Imidazole	500 mM ⁴
MgCl ₂	1 M
Urea	max. 1 M
NaCl	5 M

¹ Data kindly provided by IBA GmbH, Germany, the manufacturer and IP owner of the StrepTactin ligand.

² The additives have been successfully tested for purifying GAPDH Strep(III) 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(III)-tag in the context of the particular protein, the possible concentration may deviate from the given value for other proteins.

³ Yield may decrease.

⁴ 500 mM imidazole in sample tested by GE Healthcare.

2 General considerations

The Strep(II)-tag is a small tag of only 8 amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) and it has a molecular weight of 1 kDa. 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 StrepTactin. In addition, the optimal binding partner has been found in combination with the Strep(II)-tag. Purification is done under physiological conditions, which together with mild elution by desthiobiotin (a specific competitor that displaces the Strep(II)-tagged 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 Section 4.

3 Column packing

StrepTactin High Performance is supplied preswollen in 20% ethanol.

Prepare a slurry by decanting the 20% ethanol solution and replacing it with distilled water in a ratio of 75% settled medium to 25% distilled water. Water is used as packing solution.

Table 3. Recommended lab-scale columns for StrepTactin Sepharose High Performance

Empty Column ¹	Packing flow rate ² (ml/min)		Recommended flow rate ² for chromatography (ml/min)
	First step	Second step	
Tricorn™ 5/20	0.5	1	0.5
Tricorn 5/50	0.5	1	0.5
Tricorn 10/20	2	4	2
Tricorn 10/50	2	4	2
Tricorn 10/100	2	4	2
XK 16/20	5	10	5
XK 26/20	13	27	13

¹ For inner diameter and maximum bed volumes and bed heights, see Section 9.

² The recommended flow rates equals a linear flow rate of approximately 150 cm/h.

Packing protocol

- 1 Assemble the column (and packing reservoir if necessary).
- 2 Remove air from the end-piece and adapter by flushing with water. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.
- 3 Resuspend the medium and pour the slurry into the column in a single continuous motion.
Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.
- 4 If using a packing reservoir, immediately fill the remainder of the column and reservoir with water. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

- 5 Open the bottom outlet of the column and set the pump to run at the desired flow rate, see Table 3 or below. It is recommended to pack Sepharose High Performance media in XK or Tricorn columns in a two-step procedure. Do not exceed 1.0 bar (0.1 MPa) in the first step and 3.5 bar (0.35 MPa) in the second step.
 - If the packing equipment does not include a pressure gauge, use a first step packing flow rate of 5 ml/min (XK 16/20 column) or 2 ml/min (Tricorn 10/100 column), and a second step packing flow rate of 9 ml/min (XK 16/20 column) or 3.6 ml/min (Tricorn 10/100 column). See Table 3 for packing flow rates for other columns.
 - If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate your pump can deliver. This should also give a well packed bed.

Note: *For subsequent chromatography procedures, do not exceed 75% of the packing flow rate. See Table 3 for flow rates for chromatography.*
- 6 Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.
- 7 Stop the pump and close the column outlet.
- 8 If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.
- 9 With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.
- 10 Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

4 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. For example, 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

Recommended linear flow rate is 150 cm/h.

- 1 Remove the stoppers and connect the column to the system. Avoid introducing air into the column.
- 2 If the column has been stored in 20% ethanol, wash out the ethanol with at least 5 column volumes (CV) of distilled water or binding buffer at a linear flow rate of 50-100 cm/h.
- 3 Equilibrate the column with 5 CV of binding buffer.
- 4 Apply the pretreated sample.
- 5 Wash with 5 to 10 CV of binding buffer or until no material appears in the effluent.
- 6 Elute with ~6 CV of elution buffer. The eluted fractions can be buffer exchanged using for example a HiTrap Desalting, HiPrep 26/10 Desalting or Desalting PD-10 column.

Regeneration and cleaning

Recommended linear flow rate is 75-150 cm/h.

- 1 Regenerate and clean the column with 3 CV distilled water followed by 3 CV 0.5 M NaOH and 3 CV 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/re-equilibration is 15 CV 1 mM HABA (2-[4'-hydroxy-benzeneazo] benzoic acid) in binding buffer followed by 30 CV binding buffer. 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/StrepTactin complexes. The HABA is washed away with the binding buffer.*

Table 4. Prepacked columns for desalting and buffer exchange.

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep 26/10 Desalting	17-5087-01	2.5 to 15 ml	7.5 to 20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts ($M_r > 5000$).
	17-1408-01	0.25 to 1.5 ml	1.0 to 2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
HiTrap Desalting					
PD-10 Desalting	17-0851-01	1.0 to 2.5 ml ¹ 1.75 to 2.5 ml ²	3.5 ml ¹ up to 2.5 ml ²	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules ($M_r > 5000$).
	PD MimiTrap™ G-25	28-9180-07 0.1 to 0.5 ml ¹ 0.2 to 0.5 ml ²	1.0 ml ¹ up to 0.5 ml ²		
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml ¹ 0.75 to 1.0 ml ²	1.5 ml ¹ up to 1.0 ml ²		

¹ Volumes with gravity elution² Volumes with centrifugation

5 Scale up

Scale-up is typically performed by keeping bed height and linear flow rate (cm/h) constant while increasing bed diameter and volumetric flow rate (ml/min).

6 Storage

Store StrepTactin Sepharose High Performance in 20% ethanol at 4 to 8°C. After storage, equilibrate with binding buffer before use.

7 Troubleshooting

Fault	Possible cause/corrective action
Increased back pressure	<p>High viscosity of solutions.</p> <ul style="list-style-type: none">• <i>Use lower flow rates</i> <p>Insufficient cell disruption.</p> <ul style="list-style-type: none">• <i>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).</i>• <i>Increase dilution of the cell paste before mechanical lysis, or dilute after lysis to reduce viscosity.</i>• <i>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.</i>• <i>If the purification has been performed at 4°C, try repeating it at room temperature if possible (sample viscosity is reduced at room temperature).</i>• <i>Decrease flow rate during sample loading.</i>

Fault	Possible cause/corrective action
Increased back pressure	Freezing/thawing of the unclarified lysate has increased precipitation and aggregation. <ul style="list-style-type: none"> • <i>Centrifuge the thawed lysate.</i>
Column has clogged	Top filter is clogged. <ul style="list-style-type: none"> • <i>Change top filter.</i> Cell debris in the sample may clog the column. <ul style="list-style-type: none"> • <i>Clean the column according to the section under Operation.</i> • <i>Centrifuge and filter the sample through a 0.22 μm or a 0.45 μm filter.</i>
No or weak binding to the column	Protein found in the flow-through. <ul style="list-style-type: none"> • <i>Buffer/sample composition is not optimal; check the pH and composition of the sample and binding buffer. pH should be 7.0 or higher.</i> Strep(III)-tag is not present. <ul style="list-style-type: none"> • <i>Use protease-deficient E. coli expression strains. Add protease inhibitors during cell lysis.</i> Strep(III)-tag is not accessible <ul style="list-style-type: none"> • <i>Fuse Strep(III)-tag with the other protein terminus: Use another linker.</i> The ligand is blocked by biotinylated proteins from the extract. <ul style="list-style-type: none"> • <i>Add avidin 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.</i> Protein has precipitated in the column due to high protein concentration. <ul style="list-style-type: none"> • <i>Clean the column according to instructions under Operation. In the following run, 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.</i>

Fault	Possible cause/corrective action
Contaminating proteins	Contaminants are short forms of the tagged protein.
	<ul style="list-style-type: none"> • <i>Use protease deficient E. coli expression strains. Add protease inhibitors after cell lysis. Fuse the Strep(III)-tag with the other protein terminus. Check for the presence of internal translation initiation starts (for C-terminal Strep(III) - tag) or premature termination sites (for N-terminal Strep(III)-tag). Use EDTA in the sample and buffers.</i>
	Contaminants are covalently linked to the recombinant protein via disulfide bonds.
Unwanted air bubble formation	<ul style="list-style-type: none"> • <i>Add reducing agents to all buffers for cell lysis and purification.</i>
	Contaminants are non-covalently linked to the recombinant protein.
	<ul style="list-style-type: none"> • <i>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).</i>
Unwanted air bubble formation	Unclarified lysates may increase air bubble formation during purification.
	<ul style="list-style-type: none"> • <i>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 adjust for the extra pressure from the flow restrictor. Do not exceed the pressure limit for the column on the ÄKTA design™ system</i>
	Air bubbles may form due to decreased air solubility when columns stored at 4 to 8°C are used immediately at room temperature.
Unwanted air bubble formation	<ul style="list-style-type: none"> • <i>Let the columns adapt to room temperature for some minutes before using them.</i>

8 Further information

Refer to IBA GmbH, Germany, www.iba-go.com, for expression, detection and/or assays for Strep(II)-tagged proteins.

For further information, visit www.gelifesciences.com/protein-purification or our technical support portal www.gelifesciences.com/purification_techsupport or your local GE Healthcare representative.

9 Ordering Information

Product	Pack size	Code No.
StrepTactin Sepharose High Performance	10 ml	28-9355-99
StrepTactin Sepharose High Performance	50 ml	28-9356-00

Related products	Pack size	Code No.
StrepTrap HP	5 × 1 ml	28-9075-46
StrepTrap HP	1 × 5 ml	28-9075-47
StrepTrap HP	5 × 5 ml	28-9075-48
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml ¹	11-0003-29
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02
PD-10 Desalting Column	30	17-0851-01
PD MiniTrap G-25	50	28-9180-07
PD MidiTrap G-25	50	28-9180-08

¹ Special pack size delivered on specific customer order.

Empty lab-scale columns	Pack size	Code No.
Tricorn 5/20 column, 5 mm i.d., max 0.55 ml bed volume or 2.8 cm bed height	1	18-1163-08
Tricorn 5/50 column, 5 mm i.d., max 1.1 ml bed volume or 5.8 cm bed height	1	18-1163-09
Tricorn 10/20 column, 10 mm i.d., max 2.2 ml bed volume or 2.8 cm bed height	1	18-1163-13
Tricorn 10/50 column, 10 mm i.d., max 4.5 ml bed volume or 5.8 cm bed height	1	18-1163-14
Tricorn 10/100 column, 10 mm i.d., max 8.5 ml bed volume or 10.8 cm bed height	1	18-1163-15
XK 16/20 column, 16 mm i.d., max 30 ml bed volume or 15 cm bed height	1	18-8773-01
XK 26/20 column, 26 mm i.d., max 65 ml bed volume or 12.5 cm bed height	1	18-1000-72
Related literature		Code No.
Affinity Chromatography Handbook, Principles and Methods		18-1022-29
Affinity Chromatography Column and Media, Selection Guide		18-1121-86
Recombinant Protein Purification Handbook, Principles and Methods		18-1142-75

For contact information for your local office, please visit:
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/protein-purification

GE Healthcare Europe GmbH
Munzinger Strasse 5,
D-79111 Freiburg,
Germany

GE Healthcare UK Ltd
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue
P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg.
3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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