Streptavidin Sepharose High Performance

Purified Streptavidin isolated from *Streptomyces avidinii* is immobilised on Sepharose™ High Performance. The base matrix is a rigid, highly cross-linked beaded agarose with high chemical stability. The immobilised streptavidin binds biotin and biotinylated substances and can be used for affinity chromatography applications. The interaction between streptavidin and biotin is very strong and requires denaturating 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 Streptavidin Sepharose High Performance from which the antigen can be eluted.

Another example is to utilise the interaction between 2-iminobiotin and streptavidin, eluting the bound substances at pH 4.

Streptavidin Sepharose High Performance is also available in convenient prepacked HiTrap™ Streptavidin HP 1-ml columns.

Table 1 (see page 3) lists the characteristics of Streptavidin Sepharose High Performance.

Sepharose™

Contents

Table 1. Streptavidin Sepharose High Performance characteristics.

Ligand	Streptavidin
Binding capacity	Biotin > 300 nmol/ml medium Biotinylated serum albumin 6 mg/ml medium
Mean particle size	34 µm
Bead structure	highly cross-linked spherical agarose, 6%
Maximum back pressure	0.3 MPa (3 bar, 43 psi)
Recommended linear flow rate*	150 cm/h (equilibration and wash) 15-75 cm/h (sample application)
pH stability	
Short term	2–10.5
Long term	4–9
Temperature stability	
Short term	4°C to room temperature
Long term	4 to 8°C
Storage buffer	20% ethanol

* Linear flow rate = $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$

1 Preparing the chromatography medium

Streptavidin Sepharose High Performance is supplied pre-swollen in 20% ethanol. Wash the required amount of medium with 10 volumes of binding buffer to remove the ethanol solution. Prepare a slurry with binding buffer in a ratio of 75% settled medium to 25% buffer.

2 Packing Streptavidin Sepharose High Performance

1. Equilibrate all material at the temperature at which the chromatography will be performed.
2. De-gas the medium slurry.
3. Eliminate air from the column dead spaces by flushing the end pieces with binding buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimetres of binding buffer remaining in the column.
4. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimise the introduction of air bubbles.
5. Immediately fill the remainder of the column with binding buffer. Mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run flow rate.

3 Using an adaptor

Adaptors should be fitted as follows

1. After the chromatography medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with distilled water to form an upward meniscus at the top.
2. Insert the adaptor an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump and column and the sample application system.
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adaptor on the medium surface as necessary.

4 Binding of biotin or biotinylated substances

A specific example is the purification of antigens, where biotinylated antibodies are incubated with antigen. The biotinylated antibody-antigen complex binds to Streptavidin Sepharose High Performance and the antigen can be eluted.

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 buffer:** 20 mM sodium phosphate, 0.15 M NaCl, pH 7.5
- **Elution buffer:** 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. The sample should be filtered through a 0.45 µm filter or centrifuged before it is applied to the column.

Method

The recommended linear flow rate for Streptavidin Sepharose High Performance is 150 cm/h.

1. Fill the pump tubing with binding buffer. To avoid introducing air to the column, connect the column "drop to drop" to the pump tubing.
2. Equilibrate the column with 5 column volumes of binding buffer.
3. Apply the sample. For best results use a low flow rate, 15–75 cm/h, during sample application.

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

5 Elute with 10–20 column volumes of elution buffer. To protect thesample, adjust the pH of the eluate immediately by, for

6 example, buffer exchange on desalting columns such as a PD-10 column, HiTrap Desalting or HiPrep™ 26/10 Desalting columns depending on sample volume.

The harsh conditions required to break the streptavidin-biotin bond may affect both the sample and the ligand. Streptavidin Sepharose High Performance cannot be re-used after elution under these conditions.

5 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 buffer:** 50 mM ammonium carbonate, 0.5 M NaCl, pH 10.0
- **Elution buffer:** 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. The sample should be filtered trough a 0.45 µm filter or centrifuged before it is applied to the column.

Method

The recommended linear flow rate for Streptavidin Sepharose High Performance is 150 cm/h.

1. Fill the pump tubing with buffer. To avoid introducing air into thecolumn, connect the column "drop to drop" to the pump tubing.
2. Wash the column with at 5 column volumes of binding buffer and elution buffer respectively.
3. Equilibrate the column with 5 column volumes of binding buffer.

4 Apply the sample. For best results use a low flow rate, 15–30 cm/h, during sample application.

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

6 Storage

Streptavidin Sepharose High Performance should be stored at 4 to 8°C in 20% ethanol. The affinity medium must not be frozen.

7 Further information

If you have further questions about Streptavidin Sepharose High Performance, please visit:

www.gelifesciences.com/protein-purification

GE Healthcare tecnical support portal

www.gelifesciences.com/purification_techsupport

or contact your local GE Healthcare representative.

8 Ordering information

Product	Pack size	Code No.
Streptavidin Sepharose High Performance	5 ml	17-5113-01
Related Products		
HiTrap Streptavidin HP	5 × 1 ml	17-5112-01
PD-10 Columns	30/pack	17-0851-01
HiTrap Desalting	5 x 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
Literature		
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Columns and Media, Selection Guide	1	18-1121-86

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