

Streptavidin Sepharose High Performance HiTrap Streptavidin HP

AFFINITY CHROMATOGRAPHY

Streptavidin Sepharose™ High Performance is an affinity chromatography medium designed for fast and reliable binding of biotinylated substances. The medium is very useful since any biotinylated substance bound via streptavidin can be used as a ligand for an affinity purification. Prepacked, ready to use, HiTrap™ columns offer added convenience and speed. Streptavidin Sepharose High Performance is also supplied as a preswollen medium in suspension.

- Binding of biotinylated substances
- High capacity
- Convenient to use
- Simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems

Medium characteristics

Purified streptavidin isolated from *Streptomyces avidinii* is immobilized on Sepharose High Performance. The base matrix is a rigid, highly cross-linked, beaded agarose with good flow properties and high physical and chemical stability. The immobilized streptavidin binds biotin and biotinylated substances and can be used for affinity chromatography applications.

The medium is supplied in prepacked 1 ml HiTrap columns or as 5 ml medium in suspension stored in 20% ethanol. Medium in suspension is easily packed into for example Tricorn™ 5/50 columns (see *Ordering* information for details).

Fig 1. HiTrap Streptavidin HP and Streptavidin Sepharose High Performance.

Column characteristics

HiTrap Streptavidin HP is a 1 ml column made of polypropylene, which is biocompatible with biomolecules. The top and bottom frits are manufactured from porous polyethylene. The column is delivered with a stopper on the inlet and a twist-off end on the outlet. Connectors are included for connection to different chromatography systems and other equipments.

The characteristics of HiTrap Streptavidin HP 1 ml columns and Streptavidin Sepharose High Performance are shown in Table 1.

Table 1. HiTrap Streptavidin HP and Streptavidin Sepharose High Performance characteristics

HiTrap 1 ml column

Column dimensions, i.d. × h	0.7 × 2.5 cm
Column volume	1 ml
Recommended flow rate	1 ml/min
Maximum flow rate	4 ml/min
Column hardware pressure limit	5 bar (70 psi, 0.5 MPa)

Streptavidin Sepharose High Performance

Ligand	Streptavidin
Binding capacity	Biotin, > 300 nmol/ml medium Biotinylated bovine serum albumin, 6 mg/ml medium
Average particle size	34 µm
Bead structure	Highly cross-linked spherical agarose
pH stability	
Working*	2 to 10.5
Storage	4 to 9
Temperature stability	
Working	4°C to room temperature
Storage	4°C to 8°C
Storage buffer	20% ethanol

* Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

Convenience

HiTrap columns are designed for quick and convenient use and HiTrap Streptavidin HP is no exception. A set of connectors is supplied with the column for immediate connection to syringe (Fig 2), peristaltic pump, or liquid chromatography system. Easy to follow instructions for fast start up and method optimization are also included.

For large sample amounts, several columns can easily be connected in series (backpressure will increase). The columns cannot be opened and repacked.

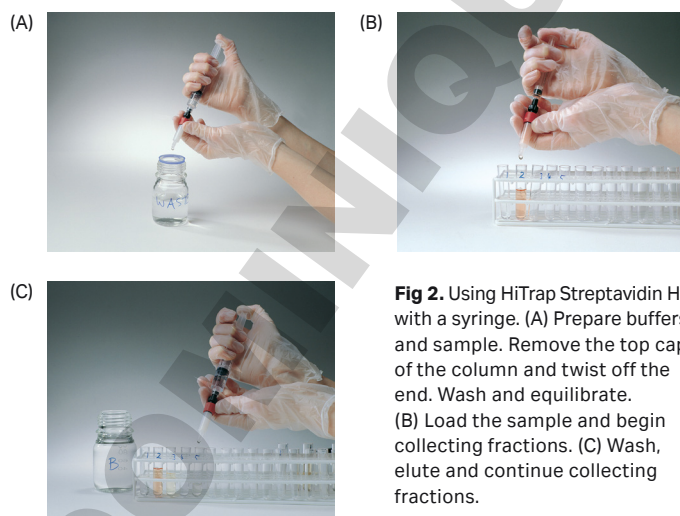


Fig 2. Using HiTrap Streptavidin HP with a syringe. (A) Prepare buffers and sample. Remove the top cap of the column and twist off the end. Wash and equilibrate. (B) Load the sample and begin collecting fractions. (C) Wash, elute and continue collecting fractions.

High capacity

As shown in Figure 3, Streptavidin Sepharose High Performance has high binding capacity and binds up to 6 mg biotinylated bovine serum albumin or > 300 nmol biotin per ml gel.

Versatility

The strong bond between streptavidin and biotin forms an excellent base for coupling any biotinylated ligand to the base matrix. This gives users the possibility to design their own affinity media, and makes Streptavidin Sepharose High Performance a very useful affinity medium.

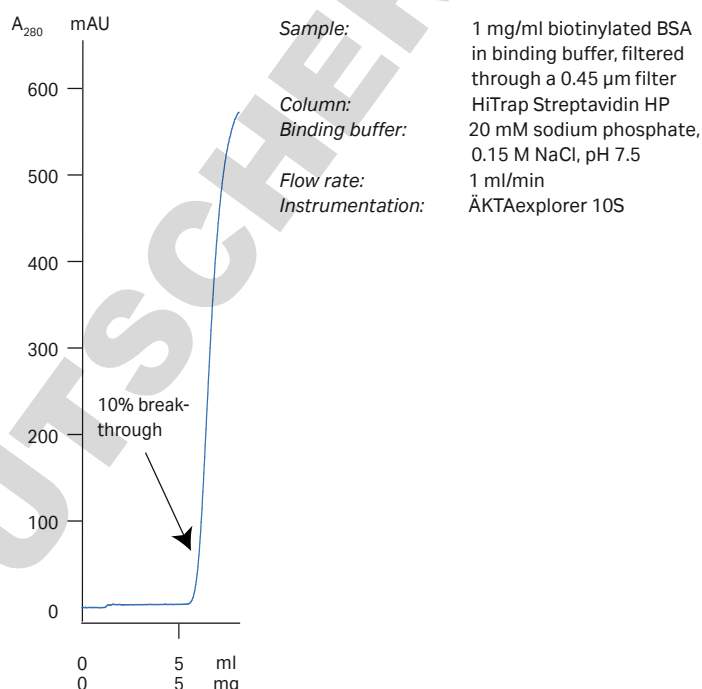
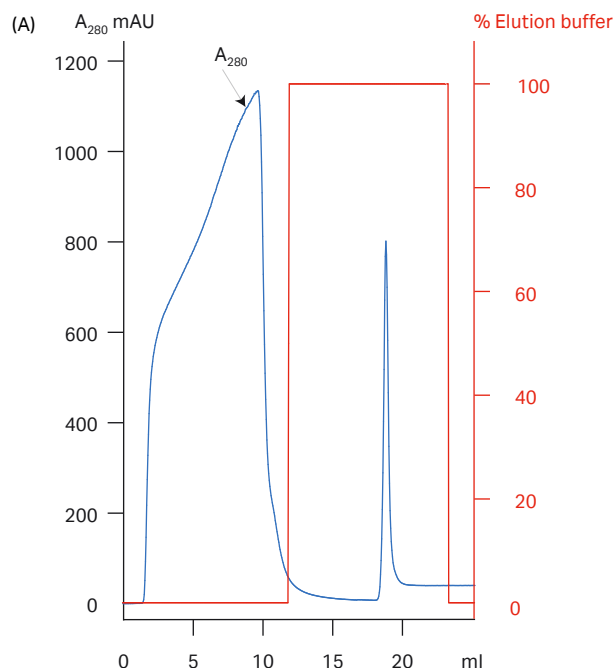


Fig 3. Binding capacity of biotinylated bovine serum albumin on HiTrap Streptavidin HP.

Applications

The interaction between streptavidin and biotin is very strong and requires denaturing conditions for elution. This strong interaction can be utilized in the purification of antigens. Biotinylated antibody-antigen complexes bind to the HiTrap Streptavidin HP column, enabling subsequent elution of the antigen. An alternative application is to exploit the interaction between 2-iminobiotin and streptavidin, which is a weaker interaction. Iminobiotinylated substances can be eluted from the column at pH 4, as shown in Figures 4A and 4B.

Sample: 9.0 ml of a mixture of BSA and iminobiotinylated BSA, filtered through a 0.45 µm filter
Column: HiTrap Streptavidin HP 1 ml
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
Flow rate: 1 ml/min (0.3 ml/min during sample application)
Instrumentation: ÄKTAexplorer 10S



Sample: 3.0 ml of eluate from A after buffer exchange on PD-10 Desalting column
Column: HiTrap Streptavidin HP 1 ml
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
Flow rate: 1 ml/min (0.15 ml/min during sample application)
Instrumentation: ÄKTAexplorer 10S

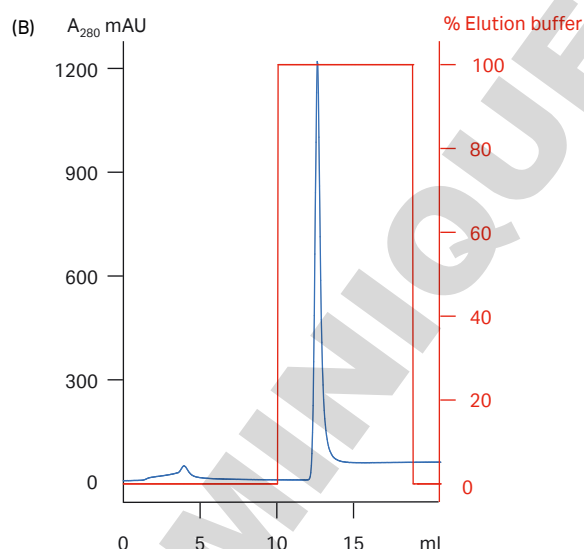


Fig 4. Purification of iminobiotinylated BSA on HiTrap Streptavidin HP.

Ordering information

Product	Quantity	Code number
Streptavidin Sepharose High Performance	5 ml	17-5113-01
HiTrap Streptavidin HP	5 × 1 ml	17-5112-01

Related products

HiTrap Desalting	1 × 5 ml	29-0486-84
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml*	11-0003-29
HiPrep™ 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02
Tricorn 5/50 column	1	18-1163-09

* Special pack size delivered on specific customer order.

Accessories	Quantity	Code number
1/16" male/Luer female*	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16" female/M6 male	6	18-1112-57
Union M6 female/1/16" male	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16"†	5	11-0004-64
Fingertight stop plug, 1/16"‡	5	11-0003-55

* One connector included in each HiTrap package

† Two, five, or seven stop plugs female included in HiTrap packages depending on products

‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery

Related literature

	Code number
Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook, Principle and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86
Convenient Protein Purification, HiTrap Column Guide	18-1129-81

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