HiTrap™ Blue HP

HiTrap Blue HP is one of a range of prepacked columns for affinity chromatography. Fast, simple and easy separations are provided by the combination of a specially designed column and a high performance affinity medium. HiTrap Blue HP is particularly suitable for the isolation and purification of albumin, interferon, a broad range of nucleotide requiring enzymes, α_2-macroglobulin, coagulation factors, and nucleic acid binding proteins. The removal of albumin is a particularly important application since excess amounts of albumin can disturb the results of several tests.

Key characteristics of HiTrap Blue HP columns are:

- Fast and convenient use
- Prepacked with Blue Sepharose™ High Performance
- Simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems.

HiTrap Blue HP is available in 1 ml and 5 ml prepacked columns that can easily be operated using a syringe. Alternatively, a laboratory pump (alone or within a chromatography system) can be advantageous, especially when linear gradients are required.

Fig 1. Prepacked with Blue Sepharose High Performance, HiTrap Blue HP columns offer fast and simple affinity purifications of proteins such as albumin, interferon, a broad range of nucleotide requiring enzymes, α_2-macroglobulin, coagulation factors, and nucleic acid binding proteins.

Medium characteristics

Sepharose High Performance is the base matrix for HiTrap Blue HP 1 ml and 5 ml columns. The carbohydrate nature of the agarose base provides a hydrophilic and chemically favorable environment for coupling, while the highly cross-linked structure of the 34 µm spherical matrix ensures good chromatographic properties. Fast kinetics and high dynamic capacities are properties of all HiTrap affinity columns.

The dye ligand, Cibacron Blue F3G-A, is covalently attached to the Sepharose High Performance matrix via the triazine part of the dye molecule. It belongs to a special class of group-specific ligands of considerable utility. Cibacron Blue F3G-A shows certain structural similarities to naturally occurring molecules such as the co-factors NAD^+ and $NADP^+$, which enables it to bind strongly and specifically to a wide range of proteins, including kinases, dehydrogenases, and most other enzymes requiring adenylyl-containing substances.

Of the enzymes currently catalogued, approximately a third requires a nucleotide coenzyme, suggesting the potential application of ligands like Cibacron Blue F3G-A to be extremely wide.

The use of immobilized dyes in place of immobilized nucleotides has a number of advantages. First, dye ligands have greater versatility, which allows purification of several proteins without tedious preparation of a range of gels. Second, the chemical stability of the adsorbent allows its continued re-use. Third, loss of activity through storage is not an issue.

Figure 2 shows the partial structure of Blue Sepharose High Performance. The main characteristics of HiTrap Blue HP columns are summarized in Table 1.

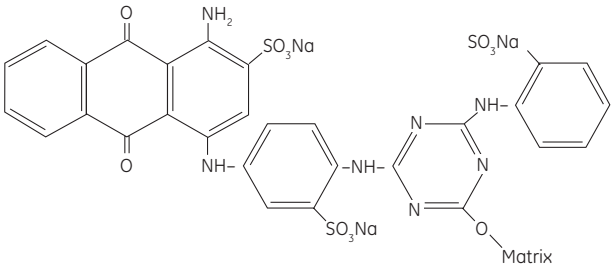


Fig 2. Partial structure of Blue Sepharose High Performance.

Table 1. Characteristics of HiTrap Blue HP

Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Ligand	Cibacron Blue F3G-A
Ligand concentration	4 mg/ml medium
Binding capacity	20 mg human albumin/ml medium
Mean particle size	34 µm
Matrix	Highly cross-linked, spherical agarose
Column hardware pressure limit	5 bar (70 psi, 0.5 MPa)
Maximum flow rate	4 ml/min (1 ml) 20 ml/min (5 ml)
Recommended flow rate	1 ml/min (1 ml) 5 ml/min (5 ml)
pH stability[1]	
Working	4 to 12
Cleaning	3 to 13
Temperature stability	
Regular use	4°C to room temperature
Storage	4°C to 8°C
Storage buffer	20% ethanol

[1] The ranges given are estimates based on our knowledge and experience. Please note the following:

pH stability, working refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

pH stability, cleaning refers to the pH interval for regeneration, cleaning-in-place, and sanitization procedures.

Column characteristics

HiTrap columns are made of polypropylene, which is biocompatible with biomolecules. Top and bottom frits are manufactured from porous polyethylene. The columns are delivered with a stopper on the inlet and a snap-off end on the outlet.

Operation

Separations on HiTrap Blue HP columns are easily performed using a syringe and the provided Luer adapter (Fig 3), a laboratory pump, or a chromatography system. Instructions and connectors are included with each pack of columns.

For quick scale-up, two or more columns can be connected in series by screwing the end of one column into the top of the next. Note, however, that connecting columns in series will cause an increase in backpressure.

(A)

(B)

(C)

Fig 3. Using HiTrap Blue HP with a syringe. (A) Prepare buffers and sample. Remove the column's top cap and snap off the end. Wash and equilibrate. (B) Load the sample and begin collecting fractions. (C) Elute and continue collecting fractions.

Applications

HiTrap Blue HP 1 ml and 5 ml can bind a very wide range of biomolecules, including: albumin, interferon, a broad range of nucleotide requiring enzymes, α_2-macroglobulin, coagulation factors, and nucleic acid binding proteins.

Some proteins combine biospecifically with Cibacron Blue due to its structural similarity to nucleotide cofactors, while others bind in a less specific manner by electrostatic and/or hydrophobic interactions with the aromatic anionic ligand. Biospecifically adsorbed proteins can be eluted by low concentrations of the free cofactor, while less specifically bound proteins require much higher concentrations of cofactor or salt.

Figure 4 shows the reliability of HiTrap columns for removal of albumin and for scaling up separations.

Scaling up the removal of albumin on HiTrap Blue HP columns

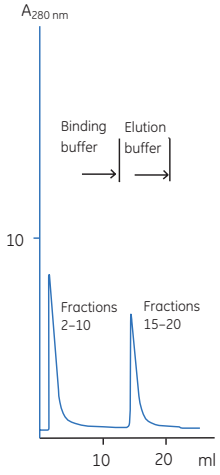
Albumin was removed from human serum in a series of experiments on six different sizes and configuration combinations of HiTrap Blue HP. The combinations were:

- HiTrap Blue HP 1 ml: 1 column, 2 columns, and 3 columns connected in series.
- HiTrap Blue HP 5 ml: 1 column, 2 columns, and 3 columns connected in series.

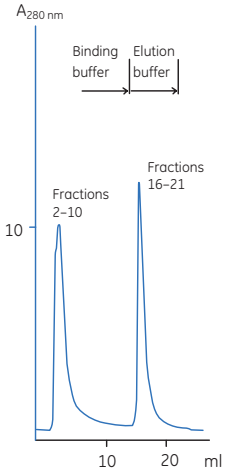
Each separation was performed with a pump. Sample and buffers were increased in proportion to the total column volume. The columns were equilibrated with the binding buffer, the sample loaded, and unbound fractions were washed out with the binding buffer and collected as pool 1. Human serum albumin was washed out with the elution buffer and fractions were collected as pool 2. The columns were then regenerated with the binding buffer.

Column: HiTrap Blue HP, 1 ml or 5 ml
Sample: Human serum buffer exchanged on a PD-10 Desalting column to the binding buffer, and filtered on a 0.45 µm filter
Flow rate: 2 ml/min (1 ml column) or 4 ml/min (5 ml column)
Binding buffer: 50 mM KH_2PO_4, pH 7.0
Elution buffer: 50 mM KH_2PO_4 with 1.5 M KCl, pH 7.0

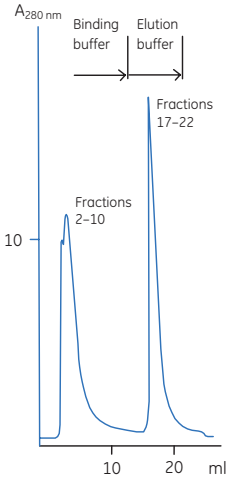
Configuration: 1 × 1 ml column
Sample volume: 0.7 ml human serum
Yield: 16.7 mg HSA



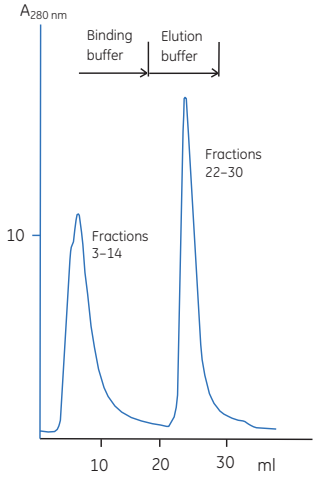
Configuration: 2 × 1 ml column
Sample volume: 1.4 ml human serum
Yield: 33.2 mg HSA



Configuration: 3 × 1 ml column
Sample volume: 2.1 ml human serum
Yield: 52.0 mg HSA



Configuration: 1 × 5 ml column
Sample volume: 3.5 ml human serum
Yield: 98.5 mg HSA



Configuration: 3 × 5 ml column
Sample volume: 10.5 ml human serum
Yield: 286.9 mg HSA

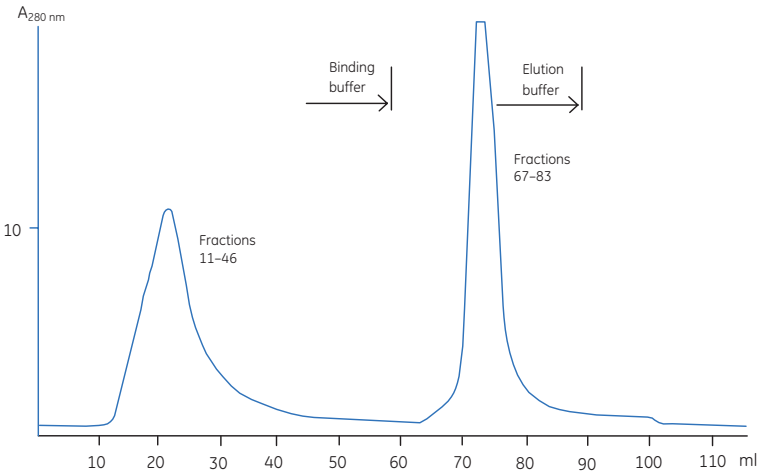


Fig 4. Scaling up a separation on HiTrap Blue HP 1 and 5 ml gives predictable separations and quantitatively reproducible yields of pure protein. The 2 × 5 ml separation, not shown, yielded 197.0 mg HSA.

Ordering information

Products	Quantity	Code number
HiTrap Blue HP	5 × 1 ml	17-0412-01
HiTrap Blue HP	1 × 5 ml	17-0413-01

Related products	Quantity	Code number
Blue Sepharose 6 Fast Flow	50 ml	17-0948-01
HiTrap Desalting	1 × 5 ml	29-0486-84
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep™ 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02

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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