cytiva

HiScreen Blue FF
HiScreen Capto Blue

Prepacked columns

Instructions for Use

HiScreen™ Blue FF and HiScreen Capto™ Blue are ready to use columns for purification of many proteins, such as albumin, interferon, lipoproteins and blood coagulation factors. They also bind several enzymes including kinases, dehydrogenases, and most enzymes requiring adenyl-containing cofactors (e.g., NAD^+).

The columns are prepacked with 4.7 ml Blue Sepharose™ 6 fast Flow and Capto Blue respectively. In Blue Sepharose 6 Fast Flow the ligand Cibacron Blue 3G is covalently attached to the base matrix by the triazine coupling method. In Capto Blue the same ligand is coupled to the base matrix by a hydrophilic spacer immobilized with a stable amine bond.

Capto Blue is more chemically stable and has a more rigid agarose base matrix than Blue Sepharose 6 Fast Flow. This allows the use of faster flow rates and larger sample volumes, leading to higher throughput and improved process economy.

The columns are ideal for screening of selectivity, binding and elution conditions, as well as small scale purifications. The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™.

Table of Contents

1 Product description 3
2 General process development 5
3 Operation 7
4 Cleaning-in-place (CIP) 12
5 Scaling up 14
6 Adjusting pressure limits 15
7 Storage 18
8 Troubleshooting 18
9 Ordering information 19

Read these instructions carefully before using the products.

Intended use

The products 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 products in a safe way, please refer to the Safety Data Sheets.

1 Product description

HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with the biomolecules. The arrow on the column label shows the recommended flow direction.

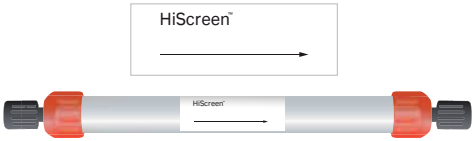

Fig 1. HiScreen column

For scale-up, when a higher bed height is required, two columns can be connected in series using a union to give a 20 cm bed height (see Section *Scaling up*).

Note: *Do not open or refill HiScreen columns.*

Note: *Check that the connector is tightened to prevent leakage.*

Table 1. Characteristics of HiScreen column

Column volume (CV)	4.7 mL
Column dimensions	0.77 × 10 cm
Column hardware pressure limit	0.8 MPa (8 bar, 116 psi)

Note: *The pressure over the packed bed varies depending on parameters such as the resin characteristics, sample/liquid viscosity, and the column tubing used.*

Properties of Blue Sepharose 6 Fast Flow and Capto Blue

The Cibacron Blue ligand contains sulfonic groups that can take part in ion exchange interactions as well as groups that can bind to the target molecule by hydrophobic interactions.

Depending on the target molecule, the effect of these groups can be enhanced or weakened by the choice of buffer salt and conductivity. To increase yield or to regenerate the chromatography resin, elution with salt can be complemented by adding an organic solvent such as ethanol or by changing pH.

The flow rate properties of the chromatography resins make HiScreen Blue FF and HiScreen Capto Blue columns ideal for establishing optimal chromatographic conditions for scaling up.

The characteristics of Blue Sepharose 6 Fast Flow and Capto Blue are summarized in the following table.

Table 2. Characteristics of Blue Sepharose 6 Fast Flow and Capto Blue

	Blue Sepharose 6 Fast Flow	Capto Blue
Matrix	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 6%, spherical
Particle size (d_{50v}) [1]	~ 90 µm	~ 75 µm
Ligand concentration	6.7 to 7.9 µmol Cibacron Blue/mL resin	11 to 16 µmol/ml Cibacron Blue/mL resin
Total binding capacity [2]	≥ 18 mg HSA/mL resin	
Dynamic binding capacity Q_{B10} [3]	~ 24 mg HSA/mL resin	
Recommended operating flow velocity (HiScreen) [4]	2.3 mL/min	2.3 mL/min

Maximum operating flow rate (HiScreen)[4]	3.2 mL/min	4.7 mL/min
pH stability, operational[5]	4 to 12	3 to 13
pH stability, CIP[6]	3 to 13	2 to 13.5
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[7] 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol	Stable to commonly used aqueous buffers, 0.01 M NaOH 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol
Storage	0.1 M KH_2PO_4, pH 8.0 in 20% ethanol, 2°C to 8°C	

1 Median particle size of the cumulative volume distribution.

2 Protein in excess is loaded in 0.050 M Potassium dihydrogen phosphate, pH 7.0 on a PEEK 7.5/50 column. The binding capacity is obtained by measuring the amount of bound and eluted protein in 0.050 M Potassium dihydrogen phosphate, 1.5 M Potassium chloride, pH 7.0.

3 Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 150 cm/h in a Tricorn™ 5/100 column at 10 cm bed height (4 min residence time) for HSA in 20 mM citric acid, pH 5.5.

4 At room temperature using buffers with the same viscosity as water. See also table *Table 4, on page 10* and *Table 5, on page 10*.

5 pH range where resin can be operated without significant change in function.

6 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

7 1.0 M NaOH should only be used for cleaning purposes.

2 General process development

HiScreen column format is ideal to use for parameter and method optimization and for robustness testing when developing a new purification process. The small column volume, 4.7 mL, and the 10 cm bed height makes it possible to perform scalable experiments at relevant process flow rates. If necessary, two columns can easily be connected in series with a union to give 20 cm bed height (see Section *Scaling up*).

The figure below outlines typical steps during general process development.

Already from start in process development it is necessary to consider process cost, cleaning of the resin, and environmental constraints.

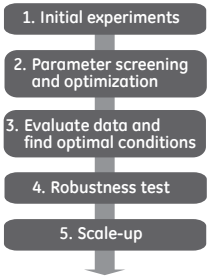

Fig 2. Typical steps during process development.

Design of Experiments (DoE) is an effective tool for investigating the effect of several parameters on protein recovery in order to establish the optimal purification protocol. See handbook *Design of Experiments in Protein Production and Purification*.A common approach in DoE is to define a reference experiment (center point) and perform representative experiments around that point. Some initial experiments are required in order to define the center point and the variable ranges. DoE may be used for parameter screening and optimization as well as robustness testing.

The robustness of a process is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. An objective of a robustness test is the evaluation of factors potentially causing variability in the responses of the method, for example, purity and yield. For this purpose, small variations in method parameters are introduced.

For scale-up, see Section *Scaling up*.

3 Operation

Prepare buffers

Start buffer

50 mM KH_2PO_4, pH 7.0

or

20 mM sodium phosphate, pH 7.0

Elution buffer

50 mM KH_2PO_4, 1.5 M KCl, pH 7.0

or

20 mM sodium phosphate, 2 M NaCl, pH 7.0

Note: *Water and chemicals used for buffer preparation must be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.*

Blue Sepharose 6 Fast Flow and Capto Blue are group specific adsorbents with affinity for a wide variety of enzymes. Some proteins interact biospecifically with the dye due to its structural similarity with nucleotide cofactors while others, such as albumin and interferon, bind in a less specific manner by electrostatic and/or hydrophobic interactions with the aromatic anionic ligand.

Biospecifically bound proteins can be eluted by low concentrations of the free cofactor, or increased ionic strength. Less specifically bound proteins require the use of much higher cofactor, or salt concentrations. Elution with cofactors normally occurs in the range 1 to 20 mM. Elution by increasing ionic strength is normally complete at salt concentrations 2 M or less (NaCl or KCl are suitable).

Binding of albumin occurs at neutral pH and elution is performed by increasing the conductivity using sodium chloride.

Note: *If not all albumin is eluted, add a hydrophobic additive to the elution buffer.*

Prepare the sample

Step	Action
1	Adjust the sample to the composition of the start buffer, using one of these methods: • Dilute the sample with start buffer. • Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap™ Desalting or PD-10 Desalting column (see table below).

Step	Action
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the life time of the column when loading large sample volumes.

Table 3. Prepacked columns for desalting and buffer exchange

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting[1]	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting[2]	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting[3]	1.0 to 2.5 mL[4]	3.5 mL
	1.75 to 2.5 mL[5]	Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL[4]	1.0 mL
	0.2 to 0.5 mL[5]	Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL[4]	1.5 mL
	0.75 to 1 mL[5]	Up to 1 mL

1 Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

2 Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

3 Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

4 Volumes with gravity elution.

5 Volumes with centrifugation.

Recommended flow rates

Table 4. Recommended flow rates using HiScreen Blue FF.

Type of operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration	≤ 3.2	≤ 410	≥ 1.5
Wash	≤ 3.2	≤ 410	≥ 1.5
Sample load	≤0.2 to 2.3	≤ 30 to 300	≥ 2 to 20
Cleaning-in-place	≤ 0.3	≤ 40	≥ 15

Table 5. Recommended flow rates using HiScreen Capto Blue.

Type of operation	Flow rate (mL/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration	≤ 4.7	≤ 600	≥ 1
Wash	≤ 4.7	≤ 600	≥ 1
Sample load	≤ 0.2 to 2.3	≤ 30 to 300	≥ 2 to 20
Cleaning-in-place	≤ 0.3	≤ 40	≥ 15

Note: *It is recommended to use a lower flow rate during sample loading and maybe also during elution due to kinetic reasons. Equilibration, wash and regeneration can usually be done at maximum flow rates if time is an issue.*

Purification

Flow rate: See *Table 4, on page 10* and *Table 5, on page 10*.

Column tubing: Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 0.25, 0.50 or 0.75 [mm]). A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.

Step	Action
1	Remove the stoppers and connect the column to the system. Avoid introducing air into the column. ***Note:*** *To prevent leakage, ensure that the connectors are tight. Use fingertight 1/16" connector (28401081).*
2	Equilibrate with at least 5 column volumes (CV) start buffer. ***Note:*** *In some cases, we recommend a blank run before final equilibration/sample application.*
3	Adjust the sample to the chosen starting conditions and load on the column.
4	Wash with 5 to 10 CV start buffer until the UV trace of the effluent returns to near baseline.
5	Elute either by linear gradient elution or a step elution at recommended flow rates. If required, the collected eluted fractions can be buffer exchanged or desalted using columns listed in *Table 3, on page 9*. • *Linear gradient elution* Elute with 0% to 100% elution buffer in 10 to 20 CV. • *Step elution* Elute with 5 CV elution buffer.

Step	Action
6	Re-equilibrate the column with 5 to 10 CV start buffer or until the UV baseline, eluent pH, and conductivity reach the required values. ***Note:*** *Do not exceed the maximum recommended flow and/or back pressure for the column.*

4 Cleaning-in-place (CIP)

General description

Correct preparation of samples and buffers maintains columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column needs cleaning.

CIP removes very tightly bound, precipitated, or denatured substances from the resin. If such contaminants are allowed to accumulate, they can affect the chromatographic properties of the prepacked column, reduce the capacity of the resin and, potentially, come off in subsequent runs. If the fouling is severe, it can block the column, increase back pressure, and affect the flow properties.

CIP must be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties, and general performance of prepacked columns.

It is recommended to perform a CIP:

- When an increase in back pressure is observed.
- If reduced column performance is observed.

- Between runs when the same column is used for purification of different proteins to prevent possible cross-contamination.
- Before first-use or after long-term storage.
- After every run with real feed.

CIP protocol

The packed column can be cleaned by the following procedures.

To remove...	Then...
Precipitated proteins	1. Wash the column with 4 column volumes (CV) of either 0.5 M (HiScreen Capto Blue) or 0.1 M NaOH (HiScreen Blue FF) at 40 cm/h. 2. Wash with 3 to 4 CV of 70% ethanol or 2 M potassium thiocyanate. 3. Wash immediately with at least 5 CV filtered start buffer, pH 8.0. or 1. Wash the column with 2 CV of 6 M guanidine hydrochloride. 2. Wash immediately with at least 5 CV filtered start buffer, pH 8.0.
Strongly bound hydrophobic proteins, lipoproteins, and lipids.	1. Wash the column with 3 to 4 CV of up to 70% ethanol or 30% isopropanol.. 2. Wash immediately with at least 5 CV filtered start buffer, pH 8.0. or 1. Wash with 2 CV detergent in a basic or acidic solution, e.g., 0.1% non-ionic detergent in 1 M acetic acid. Wash at a flow rate of 40 cm/h. 2. Remove residual detergent by washing with 5 CV of 70% ethanol. 3. Wash immediately with at least 5 CV filtered start buffer, pH 8.0.

5 Scaling up

After optimizing the method at laboratory-scale, the process is ready for scaling up.

For quick scale-up of purification, two HiScreen columns can easily be connected in series with a union (18112093) to give 20 cm bed height.

Note: *The back pressure is increased with longer bed height. This is easily addressed by lowering the flow rate.*

Other factors, such as the clearance of critical impurities can change, when column bed height is modified. The factors must be validated using the final bed height.

Scale-up to a larger column is typically performed by keeping bed height and flow velocity (cm/h) constant while increasing bed diameter and flow rate (mL/min or L/h).

BioProcess chromatography resins are developed and supported for production-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

6 Adjusting pressure limits

The pressure in chromatography system software is generated by the flow through a column. The pressure affects the packed bed and the column hardware, see the figure below. The pressure is Increased during 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 recommended flow rates in Table 2, on page 4) can damage the column.*

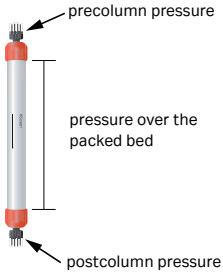

Fig 3. Precolumn and postcolumn measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (precolumn pressure and pressure over the packed bed, Δp). The precolumn pressure limit is the column hardware pressure limit (see *Table 1, on page 3* and *Table 2, on page 4*).

The maximum pressure the packed bed can withstand depends on resin 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 the optimal functionality in ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with pressure sensor in the pump, the pressure limit in the software can be adjusted as follows:

Step	Action
1	• Replace the column with a piece of tubing. • Run the pump at the maximum intended flow rate. • Record 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. • Record the pressure as P2.
3	• Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see *Table 1, on page 3*). • Replace the pressure limit in the software with the calculated value. *Result:* The actual pressure over the packed bed (Δp) during the run is equal to the actual measured pressure which is the total system pressure (P1).

Note: *Repeat the procedure each time the parameters are changed.*

7 Storage

Store HiScreen Blue FF and HiScreen Capto Blue columns equilibrated with 5 to 10 CV of 0.1 M K_2PO_4, pH 8.0 in 20% ethanol at 2°C to 8°C. Do not freeze.

Make sure that the column is tightly sealed to avoid drying out.

8 Troubleshooting

Problem	Possible cause	Corrective action
High back pressure during the run	Solutions with high viscosity are used	Use a lower flow rate.
	The column is clogged.	Clean the column, see section Cleaning-in-place (CIP)
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump.	If possible, de-gas the sample using a vacuum degasser.
Gradual broadening of the eluate peak	Insufficient elution and CIP, caused by contaminants accumulating in the column.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual decrease in yield	Insufficient elution and CIP	Optimize the elution conditions, for example, add hydrophobic additives to elution buffer. Optimize the CIP protocol and/or perform CIP more frequently.
Precipitation during elution	Suboptimal elution conditions and CIP	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.

Problem	Possible cause	Corrective action
Gradual increase in CIP peaks	Suboptimal elution conditions and CIP	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
High back pressure during CIP	Proteins are precipitated in the column.	Optimize elution conditions and/or run high salt wash before CIP or use lower flow rate.
Reduced column performance despite optimized elution and CIP	Column longevity, which depends mainly on the sample type and sample preparation.	Change to a new column.

9 Ordering information

Product	Quantity	Code No.
HiScreen Blue FF	1 × 4.7 ml	28978243
HiScreen Capto Blue	1 × 4.7 ml	28992474

Related products	Quantity	Code No
HiTrap Blue HP	5 × 1 ml	17041201
	1 × 5 ml	17041301
Blue Sepharose 6 Fast Flow	50 ml	17094801
	500 ml[1]	17094802
Capto Blue	25 ml	17544801
	500 ml[1]	17544802
HiTrap Desalting	5 × 5 ml	17140801
HiPrep 26/10 Desalting	1 × 53 ml	17508701
	4 × 53 ml	17508702

[1] Process-scale quantities are available. Please contact your local representative.

Accessories HiScreen	Quantity	Code No
HiTrap/HiPrep, 1/16" male connector for ÄKTA (*For connection of columns with 1/16" fittings to ÄKTA*)	8	28401081
Union 1/16" male/1/16" male with 0.5 mm i.d. (*For connecting two columns with 1/16" fittings in series*)	2	18112093
Fingertight stop plug, 1/16"[1] (*For sealing a HiScreen column*)	5	11000355

[1] One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

Related literature	Code No.
Affinity Chromatography Handbook, Principles and Methods	18102229
Affinity Chromatography Column and Media, Selection Guide	18112186
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28931778

Page intentionally left blank

cytiva

cytiva.com.com/protein-purification

For local office contact information, visit cytiva.com/contact

28979078 AE V:6 07/2020