GE Healthcare
Life Sciences

Instructions 28-9790-78 AC **Prepacked columns**

HiScreen™ Blue FF
HiScreen Capto™ Blue

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

Please read these instructions carefully before using the HiScreen columns.

Intended use
HiScreen columns 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 product in a safe way, please refer to the Safety data sheet.

1 Product description

HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label indicates the column orientation and the recommended flow direction, see Figure 1.

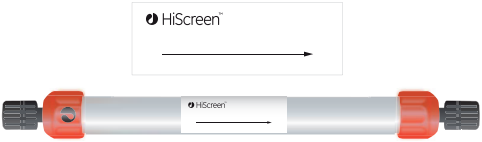

Fig 1. HiScreen column

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

Note: *HiScreen columns cannot be opened or refilled*

Note: *Make sure that the connector is tight 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 [1]	8 bar (0.8 MPa)

[1] The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium 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 medium, 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 media 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	Highly cross-linked agarose	
Average particle size (d_{50v}) [1]	90 µm	75 µm
Ligand density	11 to 16 µmol/ml	11 to 16 µmol/ml
Total binding capacity	>18 mg human serum albumin/ml medium	Aprox. 25 mg human serum albumin/ml medium
Recommended flow velocity [2]	30 to 300 cm/h	30 to 300 cm/h
Maximum flow velocity [2]	450 cm/h	600 cm/h
pH stability [3]		
pH working range	4 to 12	3 to 13
Cleaning-in-place stability	3 to 13	2 to 13.5
Storage	2°C to 8°C in 0.1 M K_2PO_4, pH 8.0 in 20% ethanol	

[1] d_{50v} is the average particle size of the cumulative volume distribution.

[2] Water at room temperature.
For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

[3] Working range: pH interval where the medium can be handled without significant change in function.
Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

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 medium and environmental constraints.

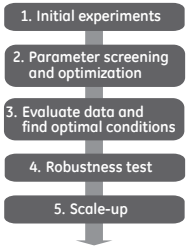

Fig 2. Typical steps during process development.

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.

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 should 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 20 to 50% ethylene glycol or another 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).
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 longevity of the column when loading large sample volumes.

Table 3. Prepacked columns for desalting

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).
HiTrap Desalting	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.	
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 MiniTrap™ 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 elution
[2] Volumes with centrifugation

Recommended flow rates

Table 4. Recommended flow rates for HiScreen Blue FF and HiScreen Capto Blue

Column	Flow velocity (cm/h)	Flow rate (ml/min)
HiScreen Blue FF	30 to 300	0.2 to 2.3
HiScreen Capto Blue	30 to 300	0.2 to 2.3

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.

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 (28-4010-81).*
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.

Step	Action
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. • *Linear gradient elution* Elute with 0% to 100% elution buffer in 10 to 20 CV. • *Step elution* Elute with 5 CV elution buffer.
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 should maintain columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column medium needs cleaning.

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

CIP should be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties and general performance of prepacked columns. CIP is usually performed after the elution.

It is recommended to perform a CIP:

- When an increase in back pressure is seen.
- If reduced column performance is observed.
- Before first time use or after long term storage.
- Between runs when the same column is used for purification of different proteins to prevent possible cross-contamination.
- 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 (18-1120-93) to give 20 cm bed height. Note that the back pressure will increase. This can easily be addressed by lowering the flow rate.

Factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height.

Scale-up 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).

Bulk media is available for further scale-up, see Section *Ordering information*.

6 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure below. Increased pressure is generated when 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 Table 2) may damage the column.*

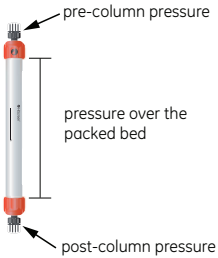

Fig 3. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media 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 optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

Step	Action
1	Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note 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. Note this pressure as P2.
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value. The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *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.

Ensure that the column is tightly sealed to avoid drying out.

8 Troubleshooting

Problem	Possible cause/*corrective action*
High back pressure during the run	The column is clogged. *Clean the column, see Section Cleaning-in-place (CIP).* High viscosity of solutions. *Use lower flow rate.*
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump. *If possible, degas 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, e.g., addition of 20 to 50% ethylene glycol or other hydrophobic additives to elution buffer. Optimize the CIP protocol and/or perform CIP more frequently*
Precipitation during elution	Sub-optimal elution conditions and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
Gradual increase in CIP peaks	Sub-optimal elution conditions and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
High back pressure during CIP	Proteins precipitated in 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	*Change to a new column. The longevity of the column depends mainly on the sample and sample preparation.*

9 Ordering information

Product	Quantity	Code No.
HiScreen Blue FF	1 × 4.7 ml	28-9782-43
HiScreen Capto Blue	1 × 4.7 ml	28-9924-74

Related products	Quantity	Code No
HiTrap Blue HP	5 × 1 ml	17-0412-01
	1 × 5 ml	17-0413-01
Blue Sepharose 6 Fast Flow	50 ml	17-0948-01
	500 ml [1]	17-0948-02
Capto Blue	25 ml	17-5448-01
	500 ml[1]	17-5448-02
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

[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	28-4010-81
Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)*	2	18-1120-93
Fingertight stop plug, 1/16" [1] *(For sealing a HiScreen column)*	5	11-0003-55

[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	18-1022-29
Affinity Chromatography Column and Media, Selection Guide	18-1121-86
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

For local office contact information, visit
www.gelifesciences.com/contact

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

www.gelifesciences.com/protein-purification

First published Oct. 2010

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

GE Healthcare UK Limited
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 Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

imagination at work

28-9790-78 AC 06/2012