Capto Q

Capto™ Q is a strong anion exchange medium for packed bed chromatography that allows increased speed and throughput in capture and intermediate purification (Fig 1). It combines high capacity with high flow velocity and low backpressure, at the same time reducing process cycle times and increasing productivity. Capto Q is a BioProcess™ medium that meets the demands of large-scale biopharmaceutical manufacturers for fast, efficient and cost-effective protein purification.

- Raised productivity with high dynamic binding capacity at high flow
- Increased yield with rapid mass transfer
- Reduced process time with high volume throughput
- Cost-effective processing with smaller unit operations

Figure 1. Capto Q increases throughput and productivity in large-scale bioprocessing operations

Media characteristics

High throughput in downstream purification requires separation media that combine mechanical strength of the matrix with a pore structure that allows fast mass transfer of target molecules. Capto Q is based on a highly rigid, chemically modified agarose. The production methodology offers outstanding pressure/flow properties with full control over the pore structure. Capto Q represents the first product in a line of new media for general-use high-flow, low-pressure solutions for bioprocessing. Table 1 summarizes the basic characteristics of Capto Q.

High flow rates and low backpressure in large-scale columns

High flow velocities increase the productivity of large-scale bioprocessing operations and allow processing of large volumes in one working shift. High flow velocities also reduce exposure of the target protein to proteases. Due to its mechanical strength and the low backpressure generated with Capto Q, columns with this medium can be operated at high flow velocities with a wide range of bed heights also in large scale. Typical flow velocities for Capto Q in a 1 m diameter column with 20 cm bed height are over 700 cm/h, with a backpressure below 3 bar.

It is important to prove pressure/flow performance in larger-scale columns where no wall support is present. Figure 2 compares pressure/flow curves in a BPG™ 300 column for the Capto base matrix with that of Sepharose™ 6 Fast Flow. Although the bead and pore sizes are similar between the two matrices, the pressure/flow properties of Capto Q are significantly better. This is a result of the exceptional mechanical stability of the Capto base matrix.

Strong anion exchanger with fast mass transfer and high dynamic binding capacity

The functional group on Capto Q is a quaternary amine (Q) group for anion-exchange. This group is linked to the matrix via a dextran surface extender.

Table 1. Characteristics of Capto Q

Matrix	highly cross-linked agarose with dextran surface extender
Ion exchange type	strong anion, Q
Charged group	$-N^+(CH_3)_3$
Total ionic capacity	0.16-0.22 mmol Cl^-/ml medium
Particle size*	90 µm (d_{50v})
Flow velocity	at least 700 cm/h in columns up to 1 m in diameter with 20 cm bed height at 20 °C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa)
Dynamic binding capacity†	> 100 mg BSA/ml medium
pH stability‡	
short term	2 – 14
working	2 – 12
long term	2 – 12
Working temperature	+4 to +30 °C
Chemical stability	all commonly used aqueous buffers 1 M acetic acid, 1 M NaOH§, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol
Avoid	oxidizing agents, anionic detergents

* d50v is the median particle size of the cumulative volume distribution.

† Dynamic binding capacity at 10% breakthrough as measured at a residence time of 1 minute, 600 cm/h in a Tricorn™ 5/100 column with 10 cm bed height in a 50 mM Tris-HCl buffer, pH 8.0.

‡ Short term pH: pH interval that the medium can be subjected to, for cleaning- or sanitization-in-place (accumulated 90–300 h at room temperature) without significant change in function.

Working pH: pH interval where the medium binds protein as intended or is needed for elution, without adverse long-term effect.

Long term pH: pH interval where the medium can be operated without significant change in function.

§ No significant change in ionic binding capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

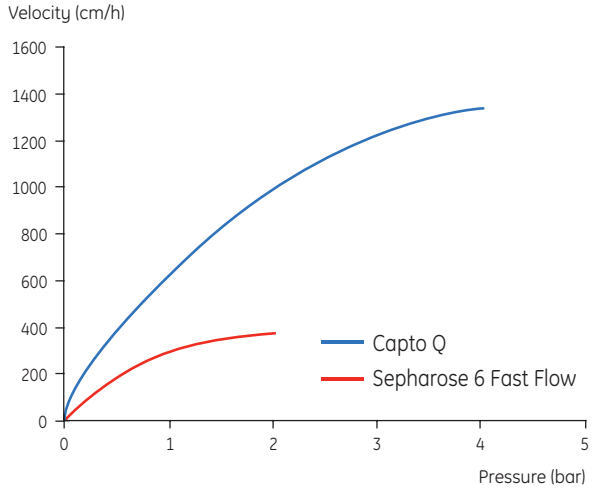


Figure 2. Pressure-flow properties of Capto Q compared to Sepharose 6 Fast Flow. Running conditions: BPG 300 (30 cm I.D.), open bed at settled bed height equal to 23 cm, with water at 20 °C.

The agarose matrix has an open pore structure that allows fast mass transfer and gives high dynamic binding capacity. The surface extender further increases capacity and mass transfer, resulting in high dynamic binding capacity also at high flow velocities. High dynamic binding capacity is important for obtaining high yield of the target protein. It also contributes to shortening the overall processing time as the total number of cycles may be reduced. Dynamic binding capacities at different residence times are shown in figure 3.

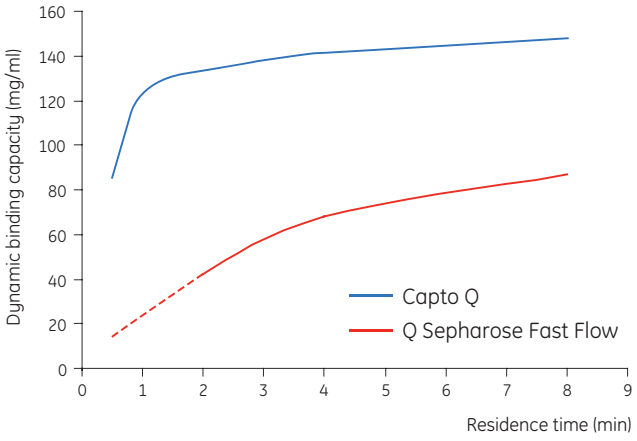


Figure 3. The agarose matrix has an open pore structure that allows fast mass transfer. Dynamic binding capacity for bovine serum albumin (BSA) is shown as a function of residence time. For Q Sepharose Fast Flow, residence times below 2 minutes can not be achieved in large-scale columns.

Agarose assures protein friendliness and high chemical stability

Agarose is a natural polymer with hydrophilic properties that minimizes structural changes of the target molecule and unspecific adsorption to the matrix. It is compatible to the broad range of buffer systems commonly used in bioprocess chromatography for protein purification. Therefore optimized conditions for binding and elution can be applied, assuring mild treatment of the target molecules.

The chemical stability of agarose prolongs media lifetime even if harsh cleaning-in-place procedures are applied. Characteristics such as capacity, elution behavior and pressure/flow properties are not affected by common cleaning solutions in process chromatography. These properties make agarose media well accepted by regulatory bodies and have resulted in proven track records in biotherapeutic manufacturing.

Rigid medium for cost-effective purification

The rigidity of the Capto Q base matrix translates into improved process economics. Its characteristics allow a wider working range of flow velocities, bed heights and sample viscosities, all of which affect processing costs in a positive way. High flow velocities increase volume throughput and reduce process time, longer bed heights means smaller equipment

and reduced foot-print, and high-flow processing of viscous samples means less dilution and shorter cycle times.

The available degree of freedom in process design for a medium can be illustrated as its "window of operation". Figure 4 shows schematically the ranges for key operating variables for Capto Q and Sepharose 6 Fast Flow. Given a maximum allowed pressure, it predicts the allowable combinations of column bed heights and operating velocities. The pressure limits, shown as blue and red curves, are based on a 1 meter diameter column with 20 cm bed height and maximum operating velocities of 700 and 250 cm/h, respectively. At this point, the pressure is 3 bar for Capto Q and 1 bar for Sepharose 6 Fast Flow. For Sepharose 6 Fast Flow, 1 bar represents the highest recommended pressure. For Capto Q, 3 bar corresponds to the maximum pressure for many low-pressure systems; the medium as such can normally be run to the maximum pressure rating of low and medium pressure columns.

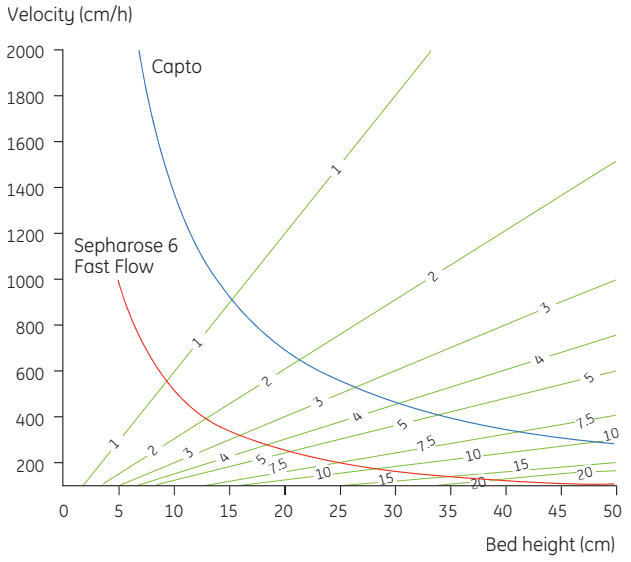


Figure 4. The highly rigid Capto base matrix allows a much larger window of operation (area below the curves) at large-scale than Sepharose 6 Fast Flow. This is particularly true at bed heights of 25-30 cm and above. Data correspond to a 1 m diameter column, 20 cm bed height, 20°C and viscosity of water. Red and blue curves correspond to pressure limits of 1 and 3 bar, respectively. Green contours give the residence time in the column in minutes.

The size of the area below the pressure limit curves represents the window of operation, or the available operating range for the respective medium. As shown in Figure 4, this is significantly larger for Capto Q than for Sepharose 6 Fast Flow based media, especially when bed heights increase to 25-30 cm or more. At these bed heights, Capto Q can still be run at flow velocities of 300-400 cm/h or more. Thus, the high mechanical stability of Capto Q allows practical and cost-effective use of smaller diameter columns.

A large window of operation also allows flexibility even if the viscosity of the feed is high. Doubling viscosity halves the operational velocity. For a feedstock with a viscosity of 2 cP at a bed height of 30 cm, the flow velocity of Capto Q is 235 cm/h compared to 80 cm/h for Sepharose 6 Fast Flow.

Figure 4 also shows contours of the residence time in the column. Residence time is important because it affects dynamic binding capacity; the longer target molecules reside in the bed, the more of them adsorb to the medium. It is possible to increase the residence time by either decreasing the flow velocity, or increasing the column bed height. For Capto Q, increasing bed heights assures longer residence times also when high flow velocities are applied.

HiTrap columns for fast screening

Time and sample can be saved in the early stages of development by using small, prepacked HiTrap™ columns to screen for the most suitable media and to develop the basic separation method (Fig 5). In combination with an automated system such as ÄKTAexplorer™ it is easy and fast to scout for optimum selectivity, pH, ionic strength etc. Capto Q is available as HiTrap prepacked 1 ml and 5 ml columns. Basic characteristics of HiTrap Capto Q columns are summarized in Table 2.

Table 2. Characteristics of HiTrap columns

Column volumes	1 ml and 5 ml
Column dimensions	0.7 x 2.5 cm (1 ml); 1.6 x 2.5 cm (5 ml)
Maximum flow rates	HiTrap 1 ml: 4 ml/min, HiTrap 5 ml: 20 ml/min
Recommended flow rates	HiTrap 1 ml: 1ml/min, HiTrap 5 ml: 5 ml/min
Maximum backpressure	0.3 MPa, (3 bar)

Application

Recent developments in upstream processing result in larger feed volumes and increased protein expression levels. The combination of high volume throughput and high capacity makes Capto Q the optimal choice for processing large amounts of protein in a fast and efficient way. As a strong anion exchanger, its behavior can be easily controlled and its application area predicted by buffer choice and pI of the target protein.

The Capto Q ligand is a quaternary amine identical to the Q ligand used in other media. Despite this, minor differences in selectivity can occur between the media as illustrated in Fig 5.

Improved productivity based on high flow features

Scale-up modeling and productivity calculations based on experimental data at small and pilot scale indicate that it is possible to capture and recover 103 kg of Green Fluorescent Protein (GFP) from an *Escherichia coli* homogenate in 24 h by using Capto Q in a 1.6 m i.d. column at 20 cm bed height. Assuming the same process cycle conditions, the corresponding amount for Q Sepharose Fast Flow is 30 kg. This example supports the argument that Capto Q as being particularly suitable for high throughput and high productivity capture purification. For further details see Application Note 11-0026-20.

Column: HiTrap, 1 ml
Sample: GFP in *E. coli* homogenate
Buffer A: 50 mM Tris/HCl, pH 8.2
Buffer B: 50 mM Tris/HCl, pH 8.2 + 1 M NaCl
Flow: 1 ml/min (156 cm/h)
Gradient: 0 – 100% B, 15 column volumes
System: ÄKTAexplorer 100

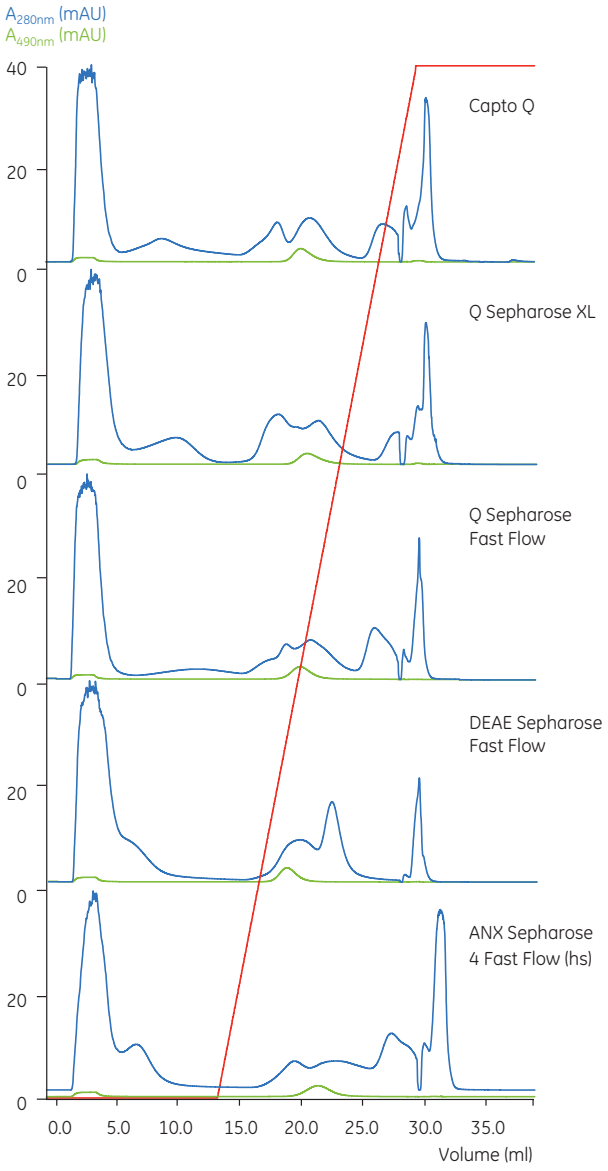


Figure 5. Five different anion exchange media available in prepacked 1 ml HiTrap columns were fast and easily screened on ÄKTAexplorer. The selectivity and separation ability for GFP, expressed in *E. coli*, on the different media were compared. GFP absorbs specifically at 490 nm and thus can be easily assayed for throughout purification. Based on the combination of the UV curves 280 nm and 490 nm Capto Q gave optimal selectivity.

Another example of how the high flow agarose technology improves throughput in capture processes is demonstrated by MabSelect™ - a protein A medium that has allowed a five-fold increase in process throughput for capture of monoclonal antibodies. The Capto Q matrix is manufactured using the same technology as MabSelect. However, the Capto matrix is even more rigid than the MabSelect matrix, to allow even higher flow velocities.

Operation

Fast method development

Fast method development can easily be achieved on ÄKTAexplorer with quick initial screening on selectivity and process conditions using prepacked HiTrap columns. Further optimization and method development using Tricorn or XK columns allows straightforward scale-up. The UNICORN™ software on ÄKTAdesign™ systems makes it simple to transfer the optimized method to a production scale process system. For more information about method development and optimization, consult the handbook, "Ion Exchange Chromatography & Chromatofocusing: Principles and Methods", (11-0004-21).

Fully scalable

Capto Q belongs to the BioProcess range of media that are developed and supported for production-scale chromatography. This includes validated manufacturing methods, secure supply and Regulatory Support Files (RSF) to assist process validation and submission to regulatory authorities.

Scale-up from laboratory purification to pilot and production scales is performed by keeping the residence time constant. The residence time is equal to the bed height (cm) divided by the flow velocity (cm/h) applied during sample load. Thus it is possible to scale-up to higher bed heights, provided that the different scales are operated with the same residence time.

This flexibility during scale-up/scale-down is a clear advantage, as lab-scale experiments are often performed with short bed heights to reduce time and sample volume. To utilize the full potential of Capto Q, we recommend bed heights of 20 cm and higher in large scale.

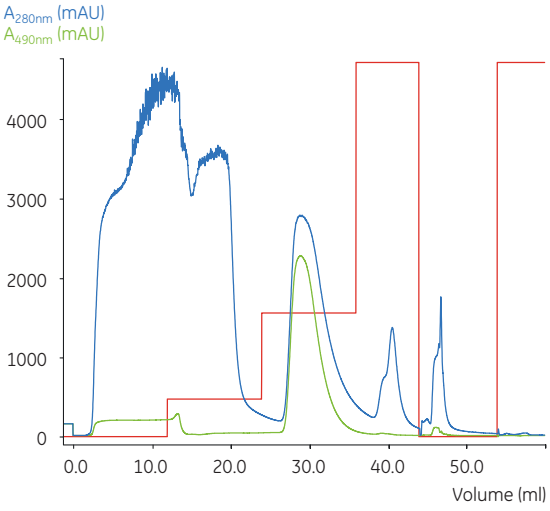
Figure 6 shows scale-up of capture purification of recombinant GFP, expressed in *E. coli* on Capto Q. Process development and optimization was performed in a Tricorn 5/100 column with 10 cm bed height. The process was scaled-up 400 times to pilot scale in a FineLINE™ 70 column with 20 cm bed height. The two-fold increase in bed height was accompanied by a two-fold increase in flow velocity, keeping the residence time constant between the scales. GFP was eluted in ~2.5 CV in FineLINE-scale, where the recovery exceeded 90%.

Cleaning and sanitization

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow properties and general performance of Capto Q.

A

Column: Tricorn 5/100, 10 cm bed height (column volume 2 ml)
Medium: Capto Q
Sample: GFP in *E. coli* homogenate, 12 ml
Buffer A: 50 mM Tris/HCl, pH 8.2
Buffer B: 50 mM Tris/HCl, pH 8.2 + 1 M NaCl
Flow rate: 300 cm/h
Gradient: 10% B (6 CV), 33% B (6 CV), 100% B (4 CV)
System: ÄKTAexplorer 100



B

Column: FineLINE 70, 20 cm bed height (column volume 808 ml)
Medium: Capto Q
Sample: GFP in *E. coli* homogenate, 4835 ml
Buffer A: 50 mM Tris/HCl, pH 8.2
Buffer B: 50 mM Tris/HCl, pH 8.2 + 1 M NaCl
Flow rate: 600 cm/h
Gradient: 10% B (6 CV), 33% B (6 CV), 100% B (4 CV)
System: ÄKTApilot™

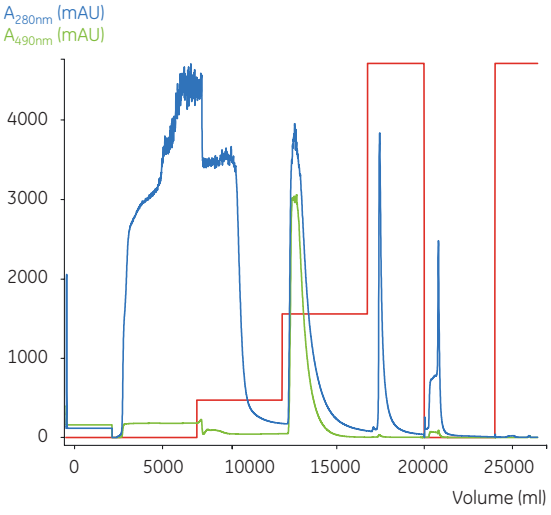


Figure 6. Straightforward scale-up between A. Tricorn 5/100 and B. FineLINE 70 (400 times).

A specific CIP protocol should be designed for each process according to the type of contaminants present. The frequency of CIP depends of the nature and the condition of the starting material, but one CIP cycle is generally recommended every 1–5 separation cycles.

Capto Q withstands all standard CIP procedures, e. g. 1 M NaOH, 2 M NaCl or 70% ethanol.

Equipment

Capto Q can be used together with most equipment available for chromatography from lab scale to production scale. Due to the high rigidity of the medium, packing procedures are slightly different compared to Sepharose 6 Fast Flow based media. For details on packing lab scale columns, see Instruction manual, for process scale columns see Application note 11-0026-21. Appropriate columns from GE Healthcare are shown in Table 3.

Table 3: Appropriate columns

Column family	Range (bed diameters)
Tricorn	5 mm, 10 mm
XK	16 mm, 26 mm
FineLINE	35 mm – 350 mm
BPG	100 mm – 300 mm*
BioProcess LPLC	100 mm – 1200 mm
Chromaflow™	400 mm – 2000 mm

* The pressure rating of BPG 450 is too low to use it with Capto Q.

Storage

Store unused media and prepacked columns at +4 to +30°C in 20% in ethanol.

Ordering information

Capto Q is also available as prepacked column in the HiTrap format.

Product	Pack size	Code No
Capto Q	25 ml	17-5316-10
Capto Q	500 ml	17-5316-01
Capto Q	5 L	17-5316-04
Capto Q	10 L	17-5316-05
Capto Q	60 L	17-5316-60
HiTrap Capto Q	5x1ml	11-0013-02
HiTrap Capto Q	5x5ml	11-0013-03

All bulk media and prepacked column products are supplied in 20% ethanol. For more information, contact your local GE Healthcare representative.

Literature

Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11-0004-21
Capture of Green Fluorescent Protein on Capto Q – application note	11-0026-20
Methods for packing Capto Q in production-scale columns - application note	11-0026-21

Further information

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