Capto™ S, Capto Q, and Capto DEAE

Capto S, Capto Q and Capto DEAE are, respectively, strong cation, strong anion and weak anion exchange media for packed bed chromatography that increase speed and throughput in capture and intermediate purification. They combine high capacity with high flow velocity and low backpressure to reduce process cycle times and increase productivity. As BioProcess™ media, the Capto range meets the demands of large-scale biopharmaceutical manufacturers by:

- Raising productivity with high dynamic binding capacity at high flow
- Reducing process time with high volume throughput
- Cost-effective processing with smaller unit operations

Chromatography 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 and high capacity for target molecules. Capto media are based on a highly rigid agarose base matrix that offers outstanding pressure/flow properties, optimized pore structure, and very high chemical stability to support CIP procedures. Capto media are intended for general use in large-scale bioprocess operations. The basic characteristics of Capto S, Capto Q, and Capto DEAE are summarized in Table 1.

High flow and low backpressure in large-scale columns

High flow velocities allow increased productivity of large-scale bioprocessing operations and processing of larger volumes in one working shift. Shorter cycle times also reduce exposure of the target protein to proteases. Typical flow velocities for Capto media in a 1 m diameter column with 20 cm bed height are over 700 cm/h, with a backpressure below 3 bar.

Figure 1 compares the pressure/flow performance of Capto with Sepharose 6 Fast Flow in a representative large-scale situation with a 1 m column that gives negligible wall support.

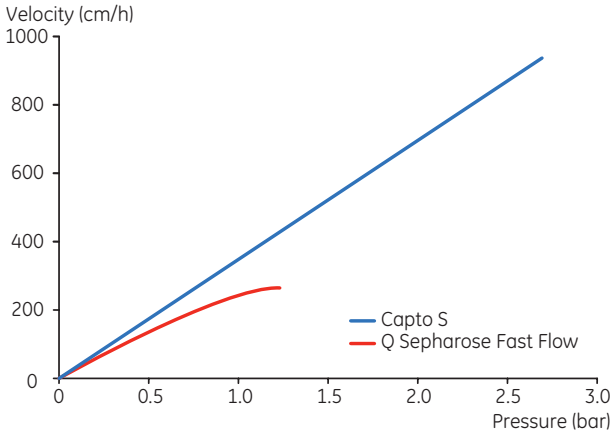


Fig 1. Pressure/flow curve for Capto S compared to Q Sepharose™ Fast Flow. *Running conditions*: AxiChrom™ 1000 for Capto S, Chromaflow™ 1000 for Q Sepharose Fast Flow, 20 cm packed bed, with water at 20°C. The pressure includes pressure drop from the bed and the column. System/tubing pressure is excluded.

Although the bead and pore sizes are similar between the two matrices, the pressure/flow properties of Capto are significantly better. This is a result of the exceptional mechanical stability of the Capto base matrix.

Anion and cation exchangers with fast mass transfer and high dynamic binding capacities

For ion exchange, Capto S uses a sulfonate group, Capto Q uses a quartenary amine group and Capto DEAE uses a diethylaminoethyl group. The groups are linked to a high flow agarose base matrix modified with a dextran surface extender which further increases capacities and mass transfer properties. Fast mass transfer ensures high dynamic binding capacity over a wide range of residence times. High binding capacity also contributes to shortening the overall processing time as the total number of cycles may be reduced. The dynamic binding capacities of Capto S, Capto Q and Capto DEAE at different residence times are shown in Figure 2.

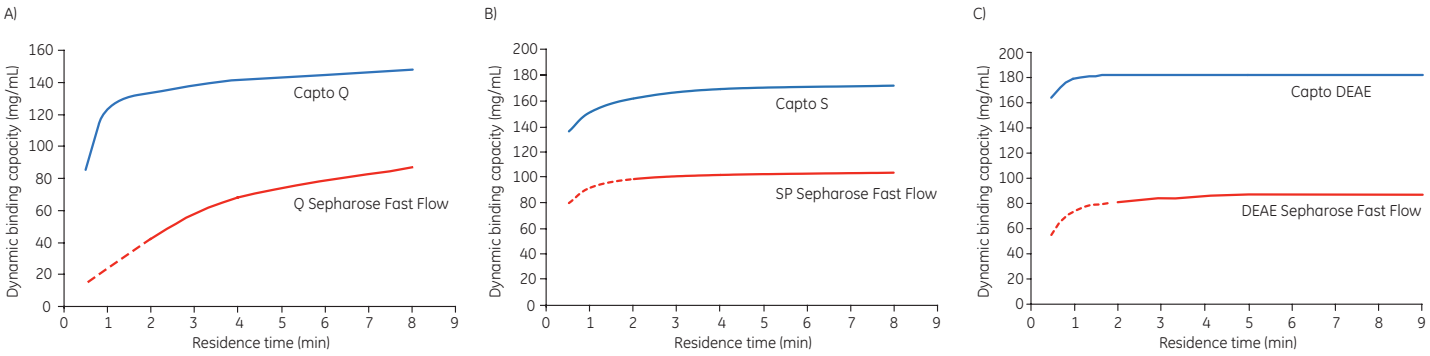


Fig 2. Dynamic binding capacity as a function of residence time for: A) Capto Q and bovine serum albumin (BSA), B) Capto S and α-chymotrypsin, C) Capto DEAE and amyloglucosidase. For Sepharose Fast Flow media, residence times below 2 min are not possible in large-scale columns.

Table 1. Characteristics of Capto S, Capto Q, and Capto DEAE

	Capto S	Capto Q	Capto DEAE
Matrix	highly cross-linked agarose with dextran surface extender		
Ion exchange type	strong cation, S	strong anion, Q	weak anion, DEAE
Charged group	$-SO_3^-$	$-N^+(CH_3)_3$	$-N^+H(CH_2CH_3)_2$
Total ionic capacity	0.11 to 0.14 mmol Na^+/mL medium	0.16 to 0.22 mmol Cl^-/mL medium	0.29 to 0.35 mmol Cl^-/mL medium
Particle size[1]	90 µm (d_{50v})	90 µm (d_{50v})	90 µm (d_{50v})
Flow velocity	at least 700 cm/h in a 1 m diameter column 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	> 120 mg lysozyme/mL medium[2]	> 100 mg BSA/mL medium[3]	> 90 mg ovalbumin/mL medium[3]
pH stability[4]			
short term	3 to 14	2 to 14	2 to 14
working	4 to 12	2 to 12	2 to 9
long term	4 to 12	2 to 12	2 to 12
Working temperature[5]	4°C to 30°C	4°C to 30°C	4°C to 30°C
Chemical stability	all commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH[6], 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol		
Storage	20% ethanol + 0.2 M NaAc	20% ethanol	20% ethanol
Avoid	oxidizing agents, cationic detergents	oxidizing agents, anionic detergents	oxidizing agents, anionic detergents

1 d_{50v} is the median particle size of the cumulative volume distribution.

2 Dynamic binding capacity at 10% breakthrough as measured at a residence time of 1 min, 600 cm/h in a Tricorn™ 5/100 column with 10 cm bed height, in a 30 mM sodium phosphate buffer, pH 6.8.

3 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.

4 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.

5 Low temperatures can decrease capacity of Capto S and Capto DEAE.

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

Rigid media for cost-effective purification

The rigidity of Capto products allows improved process economics. Capto media 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 footprint, 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 3 shows schematically the ranges for key operating variables for Capto IEX 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 m diameter column and calculated from 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 and 1.3 bar for Sepharose 6 Fast Flow. For Sepharose 6 Fast Flow, 1.3 bar represents the highest recommended operating pressure for this medium at this scale. For Capto, 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.

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 3, this is significantly larger for Capto than for Sepharose 6 Fast Flow based media, especially when bed heights increase to 20-30 cm or more. At these bed heights, Capto can still be run at flow velocities of 300-400 cm/h or more. Thus, the high mechanical stability of Capto allows practical and cost-effective use of smaller diameter columns.

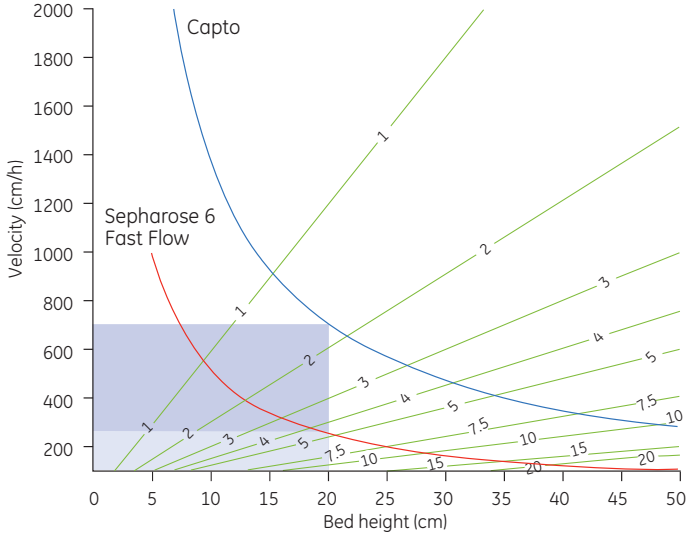


Fig 3. The highly rigid Capto base matrix allows a much larger window of operation (area below the curves) at large-scale than Sepharose 6 Fast Flow. This is particularly true at bed heights of 20-30 cm and above. Data correspond to a 1 m diameter column, at 20°C and viscosity of water. Red and blue curves correspond to pressure limits of 1.3 and 3 bar, respectively. Green contours give the residence time in the column in minutes.

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 is 235 cm/h compared to 80 cm/h for Sepharose 6 Fast Flow.

Figure 3 also shows contours of the residence time in the column. A long residence time allows for better utilization of the full equilibrium capacity. It is possible to increase the residence time by either decreasing the flow velocity, or increasing the column bed height. For Capto, increasing bed heights assures longer residence times even under high flow conditions.

Selectivity

The charged groups of the S, Q and DEAE ligands used in Capto media are identical to the charged groups used in many other ion exchange media. However, minor differences in selectivity can occur between media having the same ligand as illustrated in Figures 4 and 5. This is due to differences in base matrix, ligand density and surface extenders.

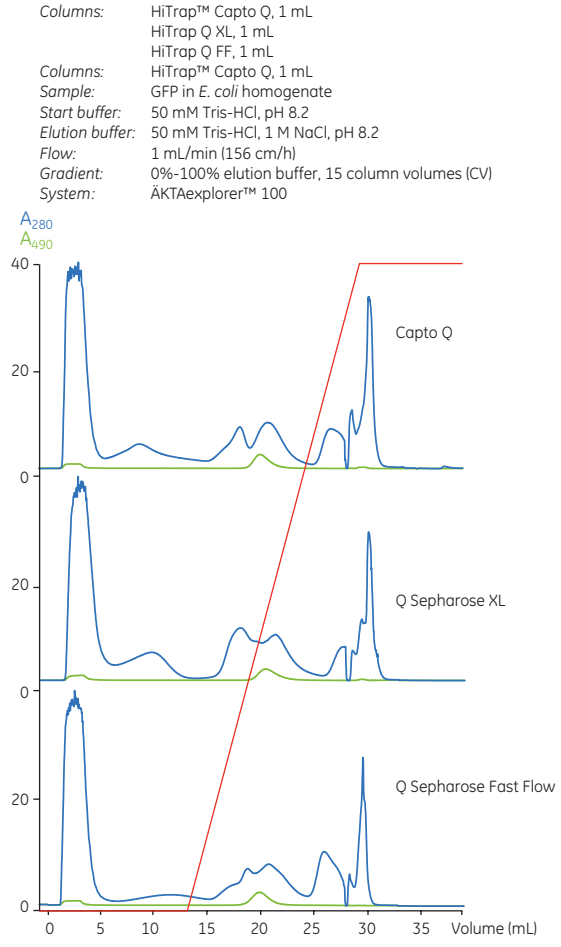


Fig 4. The separation ability of Capto Q for GFP compared to Q Sepharose Fast Flow and Q Sepharose XL media in 1 ml prepacked HiTrap columns.

Columns:	HiTrap Capto S, 1 mL HiTrap SP XL, 1 mL HiTrap SP FF, 1 mL
Sample:	α-chymotrypsin in *E. coli* homogenate
Start buffer:	50 mM Sodium acetate, pH 4.8
Elution buffer:	50 mM Sodium acetate, 1 M NaCl, pH 4.8
Flow:	1 mL/min (156 cm/h)
Gradient:	0% to 100% elution buffer, 10 CV
System:	ÄKTAexplorer 100

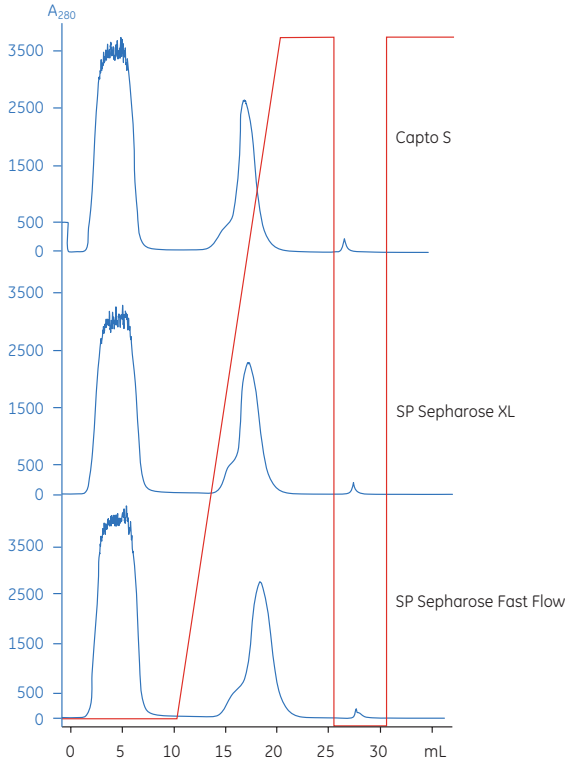


Fig 5. The separation ability of Capto S for α-chymotrypsin compared to SP Sepharose Fast Flow and SP Sepharose XL media in 1 mL prepacked HiTrap columns.

Strong vs weak ion exchangers

Strong ion exchangers like Capto S and Capto Q maintain their charge (and thus their function) over a wide pH range whereas with weak ion exchangers the degree of dissociation and thus ion exchange capacity varies with pH. Capto DEAE, although predominantly a weak anion exchanger, can not be fully discharged by raising the pH due to a minor content of quaternarized amine groups (Fig 6). It is therefore, possible to use DEAE media at higher pH values for separations of highly charged species as nucleotides, for example.

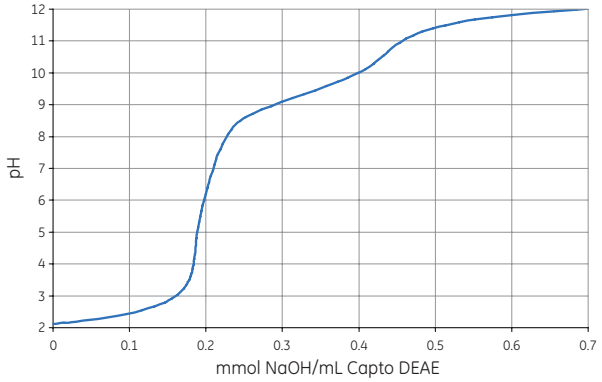


Fig 6. Titration curve of Capto DEAE.

Capto Q vs Capto DEAE

In addition to the difference in working pH range, the anion exchangers Capto Q and Capto DEAE also differ in selectivity. This difference, which is mainly pH dependent, is illustrated in Figures 7 and 8. Which product to chose depends on the individual application and what should be achieved during the separation. However the general recommendation is to start by evaluating the strong ion exchanger (Capto Q) since its function is less dependent on pH.

Column:	Tricorn 5/50, CV: 1 mL
Media:	Capto Q Capto DEAE
Sample :	Apo-transferrin (1.3 mg/mL) β-lactoglobulin (2.7 mg/mL) Pepsin (2 mg/mL)
Start buffer:	20 mM piperazine, pH 6.0
Elution buffer:	20 mM piperazine, 1 M NaCl, pH 6.0
Flow rate:	150 cm/h
Gradient:	0% to 80% elution buffer, 32 CV

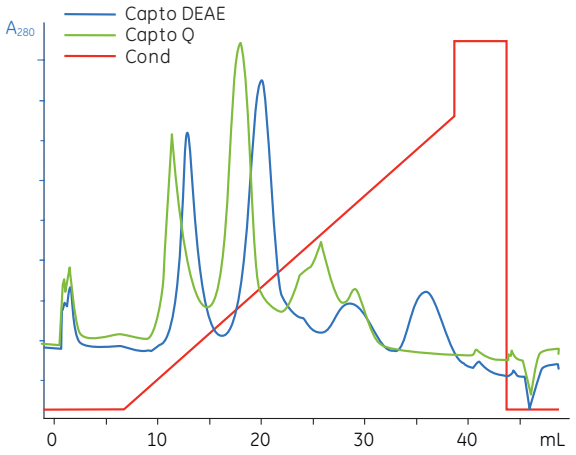


Fig 7. The selectivity difference between Capto Q and Capto DEAE at pH 6 exemplified by separation of a mixture containing three proteins and some breakdown products.

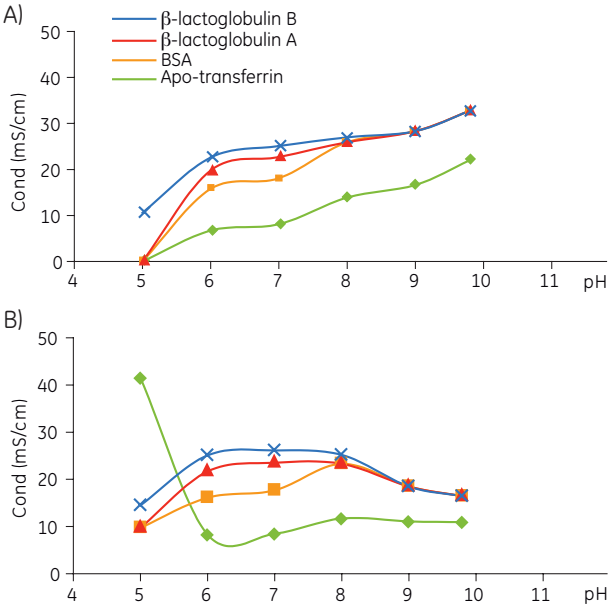


Fig 8. Elution conductivity as a function of pH for a set of model proteins on A) Capto Q, where only the change in surface charge of the proteins influences the elution position B) Capto DEAE, where both the change in surface charge of the proteins and the changed charge of the ligand determine the elution behavior.

Applications

Recent developments in upstream processing have resulted in larger feed volumes and increased protein expression levels. The combination of high volume throughput and high capacity makes Capto media the optimal choice for processing large amounts of protein in a fast and efficient way. As ion exchangers, their behavior can be easily controlled and application areas predicted by buffer choice and pI of the target proteins. Note that to reach the full potential of media where the design includes dextran, close attention must be paid to pH, conductivity, and other loading conditions. If this is done, very high capacities can be reached.

Note that the DEAE ligand has buffering properties. Therefore, a greater volume or concentration of equilibration buffer may be reqired for titration of the DEAE ligand in comparison to the non-titrateable Q ligand.

Improved productivity based on high flow features

Scale-up modelling and productivity calculations based on experimental data at small and pilot scale indicate that it is possible to capture and recover >100 kg of green fluorescent protein (GFP) from an *Escherichia coli* homogenate in 24 h using Capto Q in a 1.6 m i.d. column at 20 cm bed height (equivalent to 400 L of medium). Assuming the same process conditions, using Q Sepharose Fast Flow would require a 3 m i.d. column or 1400 L medium (in practice, this means three separate columns would be needed). This example supports the argument that Capto Q is being particularly suitable for high throughput and high productivity capture purification. For further details see Application note 11-0026-20.

Similar calculations indicate that it is possible to capture and recover >100 kg of α-chymotrypsin from *E. coli* homogenate in 24 hours with Capto S in a 0.8 m i.d. column at 20 cm bed height (equivalent to 100 L of medium). Assuming the same process cycle conditions, using SP Sepharose Fast Flow would require a 1.2 m i.d. column at 20 cm bed height (equivalent to 250 L medium). This example also indicates that Capto S is suitable for high throughput and high productivity capture purification. For further details see Application note 28-4078-15.

Process cycle times and productivity data for both examples are summarized in Table 3. Similar improvement in productivity is obtained with Capto DEAE compared to DEAE Sepharose Fast Flow.

Table 3. Results from scale-up modelling and productivity calculations for Capto Q, Capto S, and Capto DEAE as described in the text

Target protein and medium	Cycle time (min)	Productivity (kg/h, m^3)	Media volume for 100 kg/24 h (L)
GFP			
Capto Q	91	11	400
Q Sepharose Fast Flow	190	3	1400
α-chymotrypsin			
Capto S	131	53	80
SP Sepharose Fast Flow	229	17	250
amyloglucosidase			
Capto DEAE†	197	37	114
DEAE Sepharose Fast Flow†	306	12	353

† Productivity calculations based on pure protein

Operation

Fast method development

In order to find the most suitable chromatography media and/or process conditions, screening and optimization should be performed. Time and sample can be saved in the early stages of development by using small-scale formats. PreDictor™ 96-well filter plates and Assist software may be used for initial screening of process conditions such as, pH and conductivity (Fig 9). ÄKTA™ avant – with Design of Experiment (DoE) functionality - or ÄKTAexplorer chromatography systems together with prepacked columns, such as HiScreen™ and HiTrap columns, can be used for further optimization and verification of the operating conditions.

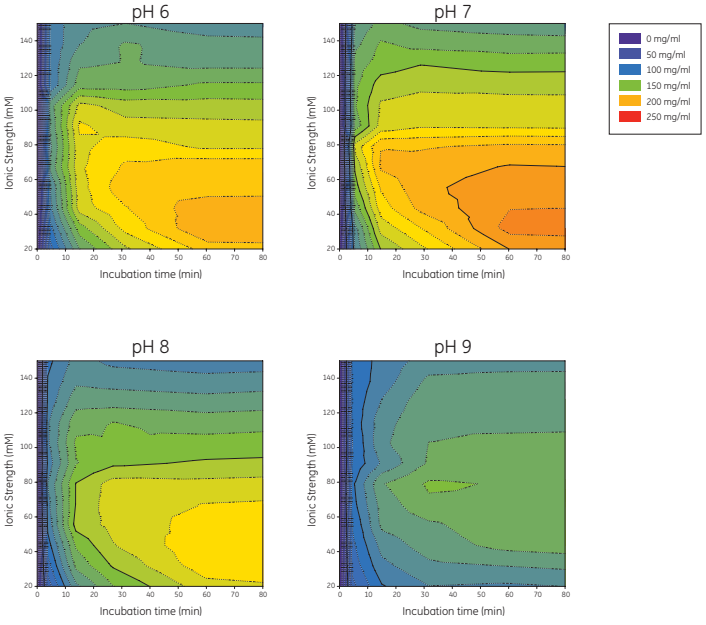


Fig 9. Screening for optimal binding conditions (pH, ionic strength and incubation time) for amyloglucosidase on Capto DEAE by batch uptake methodology using 96-well filter plates. Note that incubation time using this methodology is not equivalent to residence time in the column; typical residence times can be seen in Fig 2c.

UNICORN™ software on ÄKTA 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 handbooks, "High-throughput process development with PreDictor plates" and "Ion Exchange Chromatography & Chromatofocusing: Principles and Methods".

Fully scalable

Capto media belong 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 is typically done by keeping bed height and flow velocity constant, while increasing column bed diameter and flow rate. However, since optimization is preferentially done with small column volumes (to save sample and buffer), some parameters such as the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is constant, the binding capacity for the target molecule remains the same. Other factors, like clearance of critical impurities, may change when column bed height is changed and should be validated using the final bed height.

To utilize the full potential of Capto media, we recommend bed heights of 20 cm and higher at large scale.

A scale-up experiment was conducted for Capto S (Fig 10) using an optimized process for α-chymotrypsin with a constant residence time of 2 min. From Tricorn 5/100 the bed height was doubled to 20 cm in a XK 16/40 column (CV 40 mL). From the XK 16/40 column further scale up was conducted on an AxiChrom™ 50 (CV 400 mL) by increasing bed diameter to give a 200-fold scale up.

Cleaning and sanitization

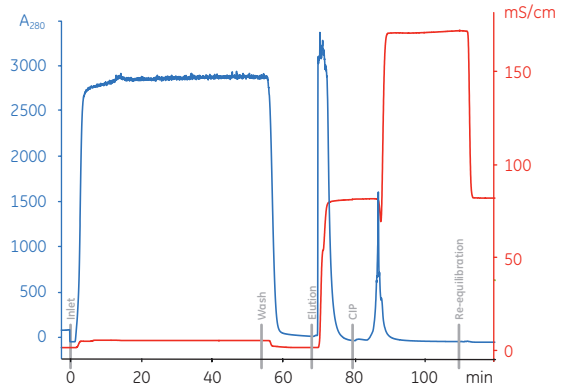
Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow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

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 to 5 separation cycles. For some contaminants a more rigorous CIP procedure can be required for Capto DEAE than for Capto S and Capto Q, see instruction manual.

All Capto media withstand all standard CIP solutions (e.g. 1 M NaOH, 2 M NaCl or 70% ethanol) or combinations thereof.

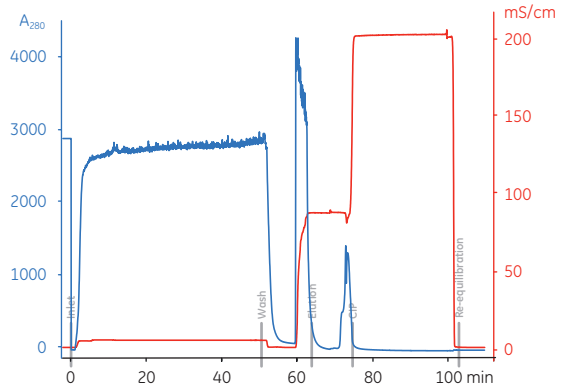
A)

Column:	Tricorn 5/100 (bed height 9.7 cm, CV=1.9 mL)
Medium:	Capto S
Sample:	α-chymotrypsin in *E. coli* homogenate, 4 mg/mL–50 mL
Start buffer:	50 mM NaAc, pH 4.8
Elution buffer:	50 mM NaAc, 1 M NaCl, pH 4.8
Flow rate:	285 cm/h
Gradient:	0%–100% 0 CV, 100% 5 CV
System:	ÄKTAexplorer 100
Residence time:	2 min



B)

Column:	XK 16/40 (bed height 20.7 cm, CV= 41.5 mL)
Medium:	Capto S
Sample:	α-chymotrypsin in *E. coli* homogenate, 4 mg/mL–1040 mL
Start buffer:	50 mM NaAc, pH 4.8
Elution buffer:	50 mM NaAc, 1 M NaCl, pH 4.8
Flow rate:	624 cm/h
Gradient:	0%–100% 0 CV, 100% 5 CV
System:	ÄKTAexplorer 100
Residence time:	2 min



C)

Column:	AxiChrom 50 (bed height 22 cm, CV= 431 mL)
Medium:	Capto S
Sample:	α-chymotrypsin in *E. coli* homogenate, 4 mg/mL–10.8 L
Start buffer:	50 mM NaAc, pH 4.8
Elution buffer:	50 mM NaAc, 1 M NaCl, pH 4.8
Flow rate:	645 cm/h
Gradient:	0%–100% 0 CV, 100% 5 CV
System:	ÄKTApilot™
Residence time:	2 min

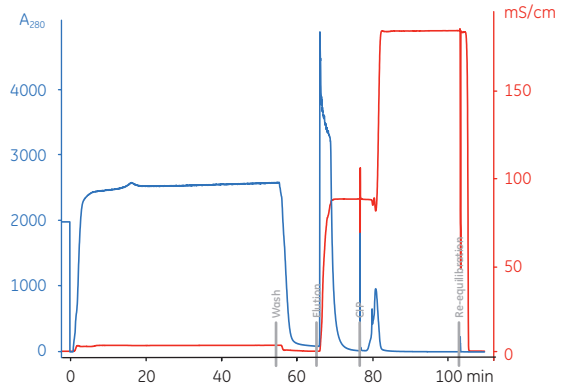


Fig 10. A 200-fold scale up using (A) Tricorn 5/100, (B) XK 16/40 and, (C) AxiChrom columns.

Equipment

Capto media 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. In process-scale, the preferred packing technique for Capto media is axial compression. Using AxiChrom columns, with Intelligent Packing and pre-set packing methods for all Capto media, is the most optimal and fastest approach. Appropriate columns from GE Healthcare are shown in Table 4. For details on packing lab-scale columns, see Instruction manuals, and for packing process-scale columns see Application notes.

All Capto media is also available in the ReadyToProcess™ platform, with pre-packed, pre-qualified and pre-sanitized ReadyToProcess columns ranging in size from 1-20 L.

Table 4. Appropriate columns

Column family range	Inner diameter (mm)
Lab scale:	
Tricorn	5, 10
HiScale™	16, 26, 50
Pilot and production scale:	
AxiChrom	50 – 1000
BPG	100 – 300†
Chromaflow	400 – 800‡

† The pressure rating of BPG 450 is too low to use it with Capto media.
‡ Larger pack stations might be required at larger diameters.

Storage

Capto S

Store unused media and prepacked columns at 4°C to 30°C in 20% in ethanol and 0.2 M sodium acetate.

Capto Q and Capto DEAE

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

Ordering information

All Capto media are available as bulk media and in several pre-packed formats, including PreDictor 96-well filter plates, PreDictor RoboColumn™, HiTrap, HiScreen, and ReadyToProcess columns. Please contact your local GE Healthcare representative for additional information.

Product	Pack size	Code no.
Capto S	25 mL	17-5441-10
Capto S	100 mL	17-5441-01
Capto S	1 L	17-5441-03
Capto S	5 L	17-5441-04
Capto S	10 L	17-5441-05
Capto S	60 L	17-5441-60
Capto Q	25 mL	17-5316-10
Capto Q	100 mL	17-5316-02
Capto Q	1 L	17-5316-03
Capto Q	5 L	17-5316-04
Capto Q	10 L	17-5316-05
Capto Q	60 L	17-5316-60
Capto DEAE	25 mL	17-5443-10
Capto DEAE	100 mL	17-5443-01
Capto DEAE	1 L	17-5443-03
Capto DEAE	5 L	17-5443-04
Capto DEAE	10 L	17-5443-05
Capto DEAE	60 L	17-5443-60
Prepacked formats		
HiTrap Capto S	5 × 1 mL	17-5441-22
HiTrap Capto S	5 × 5 mL	17-5441-23
HiTrap Capto Q	5 × 1 mL	11-0013-02
HiTrap Capto Q	5 × 5 mL	11-0013-03
HiTrap Capto DEAE	5 × 1 mL	28-9165-37
HiTrap Capto DEAE	5 × 5 mL	28-9165-40
PreDictor AIEX screening, 20 µL	4 × 96-well filter plates	28-9432-89
PreDictor AIEX screening, 2 µL/6 µL	4 × 96-well filter plates	28-9432-88
PreDictor CIEX screening 20 µL	4 × 96-well filter plates	28-9432-91
PreDictor CIEX screening 2 µL/6 µL	4 × 96-well filter plates	28-9432-90
PreDictor Capto DEAE, 2 µL	4 × 96-well filter plates	28-9258-11
PreDictor Capto DEAE, 20 µL	4 × 96-well filter plates	28-9258-12
PreDictor Capto DEAE, 50 µL	4 × 96-well filter plates	28-9258-13
PreDictor Capto DEAE Isotherm	4 × 96-well filter plates	28-9432-80
PreDictor Capto Q, 2 µL	4 × 96-well filter plates	28-9257-73
PreDictor Capto Q, 20 µL	4 × 96-well filter plates	28-9258-06
PreDictor Capto Q, 50 µL	4 × 96-well filter plates	28-9258-07
PreDictor Capto Q Isotherm	4 × 96-well filter plates	28-9432-78
PreDictor Capto S, 2 µL	4 × 96-well filter plates	28-9258-08
PreDictor Capto S, 20 µL	4 × 96-well filter plates	28-9258-09
PreDictor Capto S, 50 µL	4 × 96-well filter plates	28-9258-10
PreDictor Capto S Isotherm	4 × 96-well filter plates	28-9432-79

Product	Pack size	Code no.
PreDictor RoboColumn Capto Q, 200 µl	One row of eight columns	28-9860-72
PreDictor RoboColumn Capto Q, 600 µl	One row of eight columns	28-9861-75
PreDictor RoboColumn Capto S, 200 µl	One row of eight columns	28-9860-81
PreDictor RoboColumn Capto S, 600 µl	One row of eight columns	28-9861-76
PreDictor RoboColumn Capto DEAE, 200 µl	One row of eight columns	28-9860-82
PreDictor RoboColumn Capto DEAE, 600 µl	One row of eight columns	28-9861-77
HiScreen Capto DEAE	1 × 4.7 mL	28-9269-82
HiScreen Capto Q	1 × 4.7 mL	28-9269-78
HiScreen Capto S	1 × 4.7 mL	28-9269-79
ReadyToProcess Capto Q	2.5 L	28-9017-23
ReadyToProcess Capto Q	10 L	28-9017-24
ReadyToProcess Capto Q	1 L	28-9510-90
ReadyToProcess Capto Q	20 L	28-9017-25
ReadyToProcess Capto S	20 L	28-9017-31
ReadyToProcess Capto S	2.5 L	28-9017-29
ReadyToProcess Capto S	10 L	28-9017-30
ReadyToProcess Capto S	1 L	28-9510-93

Related literature	Code no.
Data files	
PreDictor 96-well filter plates and Assist software	28-9258-39
PreDictor RoboColumn	28-9886-34
HiScreen prepacked columns	28-9305-81
ReadyToProcess columns	28-9159-87
BPG columns	18-1115-23
Chromaflow columns	18-1138-92
AxiChrom columns	28-9290-41
Application notes	
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