Capto S, **Capto** Q, and **Capto** DEAE

ION EXCHANGE CHROMATOGRAPHY

Capto™ S, Capto Q, and Capto DEAE are, respectively, strong cation, strong anion, and weak anion exchange resins for packed bed chromatography that increase speed and throughput in capture and intermediate purification. They combine high capacity with high flow velocity and low back pressure to reduce process cycle times and increase productivity. As BioProcess™ resins, 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 resin characteristics

High throughput in downstream purification requires separation resins that combine mechanical strength of the matrix with a pore structure that allows fast mass transfer and high capacity for target molecules. Capto resins 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 resins 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 resins in a 1 m diameter column with 20 cm bed height are 700 cm/h, with a backpressure below 3 bar (0.3 MPa, 43.5 psi).

Fig 1. Capto Q, Capto S and Capto DEAE are available in multiple pack sizes for use in both lab- and production-scale applications.

Figure 2 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. Although the particle 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.

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

	Capto S	Capto Q	Capto DEAE
Matrix		Highly cross-linked agarose, spherical	
Ion exchange type	Strong cation, S	Strong anion, Q	Weak anion DEAE
Charged group	$-SO_3^-$	$-N^+(CH_3)_3$	$-N^+H(CH_2CH_3)_2$
Ionic capacity	0.11 to 0.14 mmol H+/mL resin	0.16 to 0.22 mmol Cl-/mL resin	0.29 to 0.35 mmol Cl-/mL resin
Particle size, d_{50V} [1]	~ 90 µm	~ 90 µm	~ 90 µm
Recommended maximum operating flow velocity	700 cm/h[2]	700 cm/h[2]	700 cm/h[2]
Dynamic binding capacity, Q_{B10}	> 120 mg lysozyme/mL resin[3]	> 100 mg BSA/mL resin[4]	> 90 mg ovalbumin/mL resin[4]
pH stability, operational[5]	4 to 12	2 to 12	2 to 12
pH stability, CIP[6]	3 to 14	2 to 14	2 to 14
pH ligand fully charged[7]	Entire pH range	Entire pH range	Below 9
Working temperature[8]	4°C to 30°C	4°C to 30°C	4°C to 30°C
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[9], 8 M Urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol		
Storage	20% ethanol + 0.2 M NaAc, 4°C to 30°C	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C
Avoid	Oxidizing agents, cationic detergents	Oxidizing agents, anionic detergents	Oxidizing agents, anionic detergents

[1] Median particle size of the cumulative volume distribution.
[2] 1 m diameter column and 20 cm bed height using buffers with the same viscosity as water at 20°C
[3] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 600 cm/h in an Tricorn™ 5/100 column at 10 cm bed height (1 min residence time) for Lysozyme in 30 mM sodium phosphate, pH 6.8.
[4] Dynamic binding capacity at 10% breakthough by frontal analysis at a mobile phase velocity of 600 cm/h in a Tricorn 5/100 column at 10 cm bed height (1 min residence time) for BSA/Ovalbumin in 50 mM Tris-HCl, pH 8.0.
[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] pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated or entire pH range, only use the resin within the stated stability ranges.
[8] Low temperatures can decrease capacity of Capto S and Capto DEAE.
[9] 1.0 M NaOH should only be used for cleaning purposes.

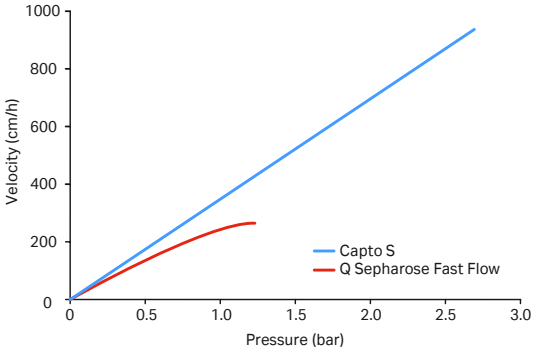


Fig 2. Pressure/flow curve for Capto S compared to Q Sepharose™ Fast Flow. Running conditions: AxiChrom™ 1000 for Capto S, Chromaflow™ 1000 for Q Sepharose Fast Flow, 20 cm packed bed, with water at 20°C. The pressure includes pressure drop from the bed and the column. System/tubing pressure is excluded.

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 can be reduced. The dynamic binding capacities of Capto S, Capto Q, and Capto DEAE at different residence times are shown in Figure 3.

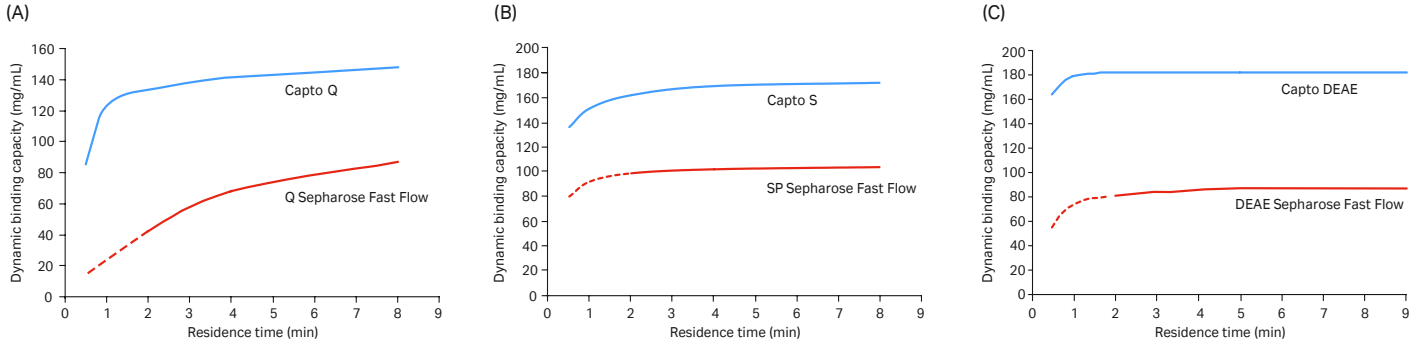


Fig 3. Dynamic binding capacity as a function of residence time for: (A) Capto Q and bovine serum albumin (BSA), (B) Capto S and α-chymotrypsin, (C) Capto DEAE and amyloglucosidase. For Sepharose Fast Flow resins, residence times below 2 min are not possible in large-scale columns.

Rigid resins for cost-effective purification

The rigidity of Capto products allows improved process economics. Capto resin 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 resin 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 resin at this scale. For Capto, 3 bar corresponds to the maximum pressure for many low-pressure systems; the resin 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 resin. As shown in Figure 4, this is significantly larger for Capto than for Sepharose 6 Fast Flow based resins, especially when bed heights increase to 20 to 30 cm or more. At these bed heights, Capto can still be run at flow velocities of 300 to 400 cm/h or more. Thus, the high mechanical stability of Capto allows practical and cost-effective use of smaller diameter columns.

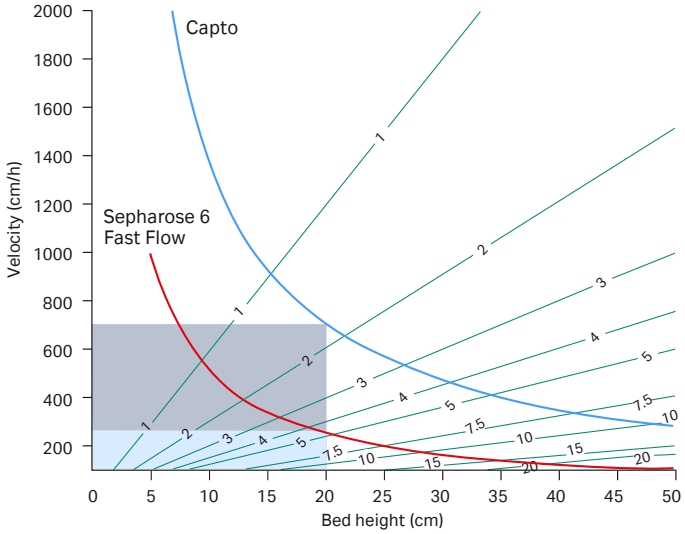


Fig 4. The highly rigid Capto base matrix allows a much larger window of operation (area below the curves) at large-scale than Sepharose 6 Fast Flow. This is particularly true at bed heights of 20–30 cm and above. Data correspond to a 1 m diameter column, at 20°C and viscosity of water. Red and blue 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 resins are identical to the charged groups used in many other ion exchange resins. However, minor differences in selectivity can occur between resins having the same ligand as illustrated in Figures 5 and 6. This is due to differences in base matrix, ligand concentration and surface extenders.

Columns:	HiTrap™ Capto Q, 1 mL HiTrap Q XL, 1 mL HiTrap Q FF, 1 mL HiTrap Capto Q, 1 mL
Sample:	GFP in *E. coli* homogenate
Start buffer:	50 mM Tris-HCl, pH 8.2
Elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 8.2
Flow:	1 mL/min (156 cm/h)
Gradient:	0%–100% elution buffer, 15 column volumes (CV)
System:	ÄKTAexplorer 100

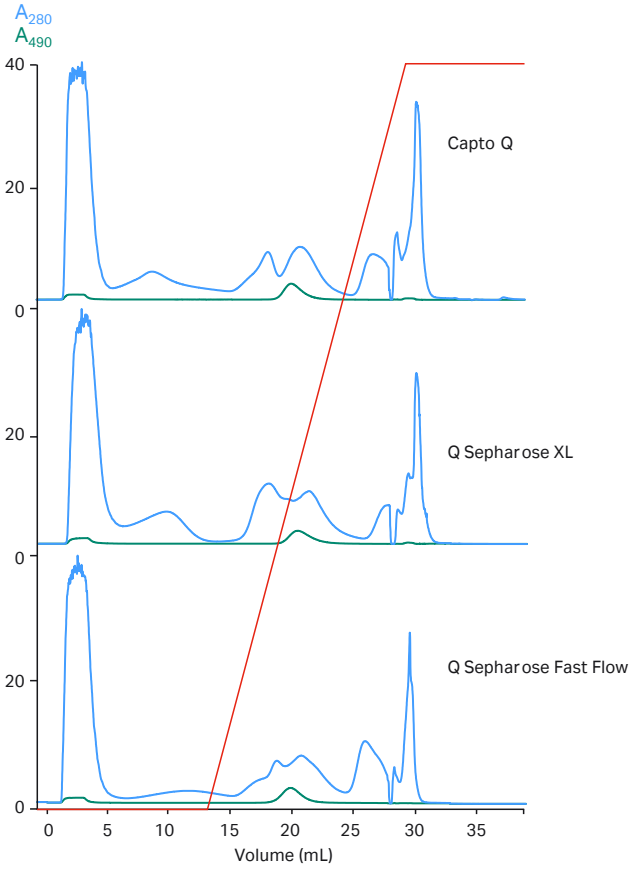


Fig 5. The separation ability of Capto Q for GFP compared to Q Sepharose Fast Flow and Q Sepharose XL resins in 1 mL prepacked HiTrap columns.

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

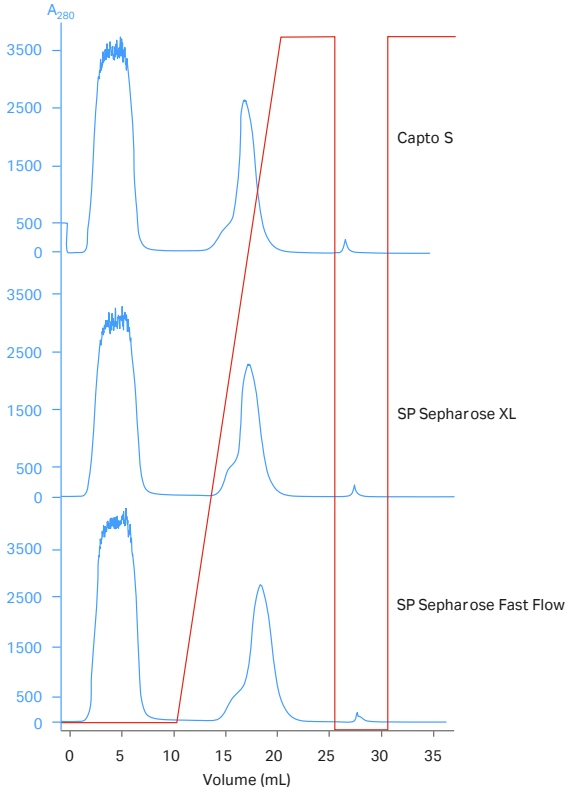


Fig 6. The separation ability of Capto S for α-chymotrypsin compared to SP Sepharose Fast Flow and SP Sepharose XL resins in 1 mL prepacked HiTrap columns.

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, cannot be fully discharged by raising the pH due to a minor content of quaternarized amine groups (Fig 7). It is therefore, possible to use DEAE resins at higher pH values for separations of highly charged species as nucleotides, for example.

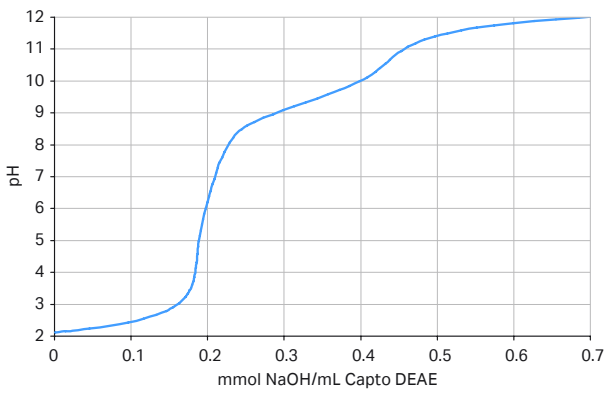


Fig 7. Titration curve of Capto DEAE.

Capto Q vs Capto DEAE

In addition to the difference in operational 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 8 and 9. Which product to choose 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
Resin: 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 velocity: 150 cm/h
Gradient: 0% to 80% elution buffer, 32 CV

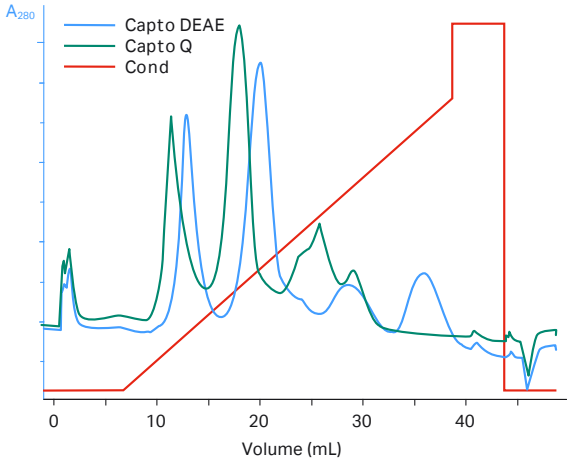


Fig 8. The selectivity difference between Capto Q and Capto DEAE at pH 6 exemplified by separation of a mixture containing three proteins and some breakdown products.

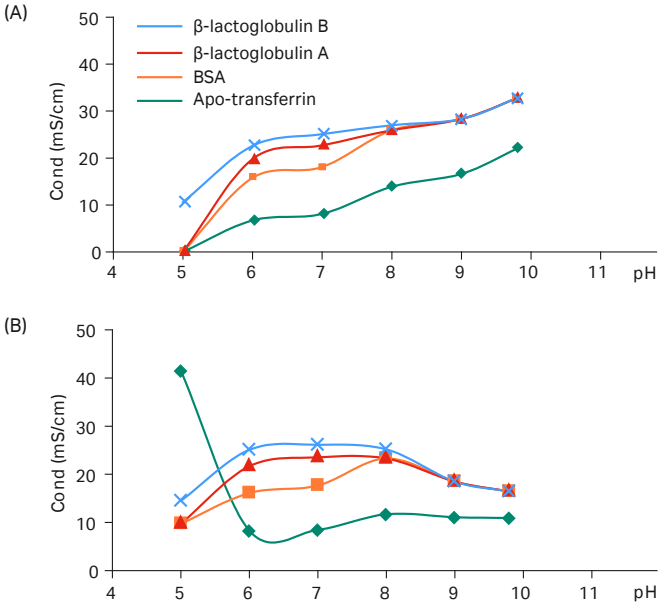


Fig 9. Elution conductivity as a function of pH for a set of model proteins on (A) Capto Q, where only the change in surface charge of the proteins influences the elution position (B) Capto DEAE, where both the change in surface charge of the proteins and the changed charge of the ligand determine the 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 resins 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 resins 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 might 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 *E. 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 resin). Assuming the same process conditions, using Q Sepharose Fast Flow would require a 3 m i.d. column or 1400 L resin (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.

Similar calculations indicate that it is possible to capture and recover > 100 kg of α-chymotrypsin from *E. coli* homogenate in 24 h with Capto S in a 0.8 m i.d. column at 20 cm bed height (equivalent to 100 L of resin). 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 resin). This example also indicates that Capto S is suitable for high throughput and high productivity capture purification.

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 resin	Cycle time (min)	Productivity (kg/h, m³)	Resin 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 resin 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 10). ÄKTA™ avant, with design of experiment (DoE) functionality chromatography system together with prepacked columns, such as HiScreen™ and HiTrap columns, can be used for further optimization and verification of the operating conditions.

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".

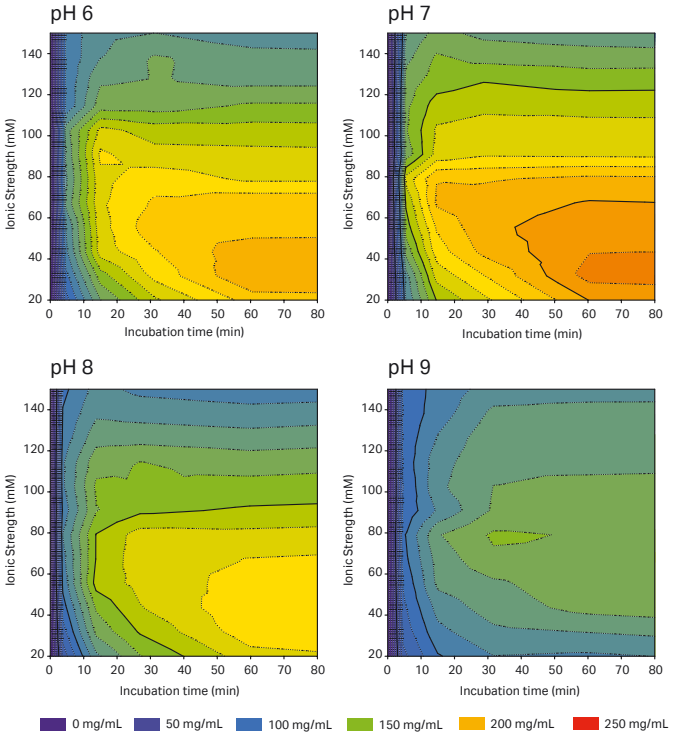


Fig 10. Screening for optimal binding conditions (pH, ionic strength and incubation time) for amyloglucosidase on Capto DEAE by batch uptake methodology using 96-well filter plates. Note that incubation time using this methodology is not equivalent to residence time in the column; typical residence times can be seen in Fig 2C.

Fully scalable

Capto media belong to the BioProcess range of resins 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, might change when column bed height is changed and should be validated using the final bed height.

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

A scale-up experiment was conducted for Capto S (Fig 11) using an optimized process for α-chymotrypsin with a constant residence time of 2 min. From Tricorn 5/100 column 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 can remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the resin bed and helps to maintain the capacity, flow properties and general performance of the resins.

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.

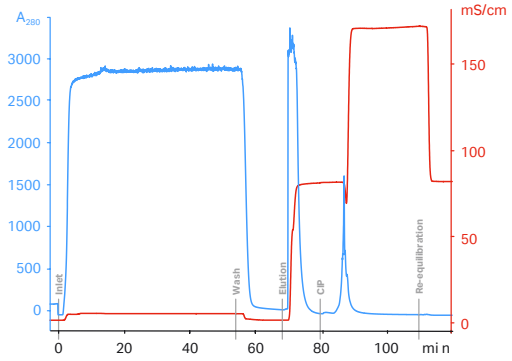
All Capto resins withstand all standard CIP solutions (e.g. 1.0 M NaOH, 2 M NaCl or 70% ethanol) or combinations thereof.

Equipment

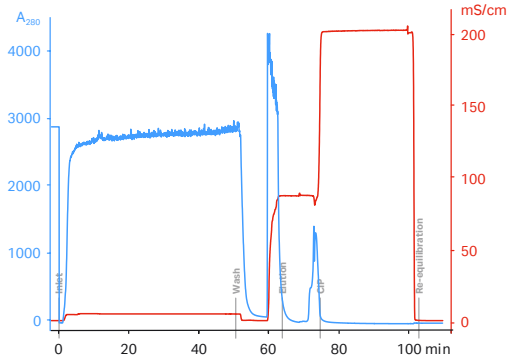
Capto resins can be used together with most equipment available for chromatography from lab scale to production scale. Due to the high rigidity of the resin, packing procedures are slightly different compared to Sepharose 6 Fast Flow based resins. In process-scale, the preferred packing technique for Capto resins is axial compression. Using AxiChrom columns, with Intelligent Packing and pre-set packing methods for all Capto resins, is the most optimal and fastest approach. Appropriate columns from Cytiva 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 resins are also available in the ReadyToProcess™ platform, with pre-packed, pre-qualified and pre-sanitized ReadyToProcess columns ranging in size from 1 to 20 L.

(A)
Column: Tricorn 5/100 (bed height 9.7 cm, CV=1.9 mL)
Resin: 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 velocity: 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)
Resin: 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 velocity: 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)
Resin: 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 velocity: 645 cm/h
Gradient: 0%–100% 0 CV, 100% 5 CV
System: ÄKTApilot™
Residence time: 2 min

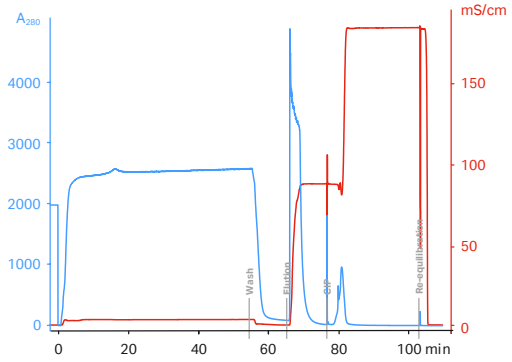


Fig 11. A 200-fold scale up using (A) Tricorn 5/100, (B) XK 16/40 and, (C) AxiChrom columns.

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 resins.
[‡] Larger pack stations might be required at larger diameters.

Storage

Capto S

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

Ordering information

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

Product	Pack size	Product code
Capto S	25 mL	17544110
	100 mL	17544101
	1 L	17544103
	5 L	17544104
	10 L	17544105
	60 L*	17544160
Capto Q	25 mL	17531610
	100 mL	17531602
	1 L	17531603
	5 L	17531604
	10 L	17531605
	60 L*	17531660
Capto DEAE	25 mL	17544310
	100 mL	17544301
	1 L	17544303
	5 L	17544304
	10 L	17544305
	60 L*	17544360
Prepacked formats		
HiTrap Capto S	5 × 1 mL	17544122
	5 × 5 mL	17544123
HiTrap Capto Q	5 × 1 mL	11001302
	5 × 5 mL	11001303
HiTrap Capto DEAE	5 × 1 mL	28916537
	5 × 5 mL	28916540
PreDictor AIEX screening, 20 µL	4 × 96-well filter plates	28943289
PreDictor AIEX screening, 2 µL/6 µL	4 × 96-well filter plates	28943288
PreDictor CIEX screening 20 µL	4 × 96-well filter plates	28943291
PreDictor CIEX screening 2 µL/6 µL	4 × 96-well filter plates	28943290
PreDictor Capto DEAE, 2 µL	4 × 96-well filter plates	28925811
PreDictor Capto DEAE, 20 µL	4 × 96-well filter plates	28925812
PreDictor Capto DEAE, 50 µL	4 × 96-well filter plates	28925813
PreDictor Capto DEAE Isotherm	4 × 96-well filter plates	28943280
PreDictor Capto Q, 2 µL	4 × 96-well filter plates	28925773
PreDictor Capto Q, 20 µL	4 × 96-well filter plates	28925806
PreDictor Capto Q, 50 µL	4 × 96-well filter plates	28925807
PreDictor Capto Q Isotherm	4 × 96-well filter plates	28943278
PreDictor Capto S, 2 µL	4 × 96-well filter plates	28925808
PreDictor Capto S, 20 µL	4 × 96-well filter plates	28925809
PreDictor Capto S, 50 µL	4 × 96-well filter plates	28925810
PreDictor Capto S Isotherm	4 × 96-well filter plates	28943279

* Pack size available upon request

Product	Pack Size	Product code
PreDictor RoboColumn Capto Q, 200 µl	One row of eight columns	28986072
PreDictor RoboColumn Capto Q, 600 µl	One row of eight columns	28986175
PreDictor RoboColumn Capto S, 200 µl	One row of eight columns	28986081
PreDictor RoboColumn Capto S, 600 µl	One row of eight columns	28986176
PreDictor RoboColumn Capto DEAE, 200 µl	One row of eight columns	28986082
PreDictor RoboColumn Capto DEAE, 600 µl	One row of eight columns	28986177
HiScreen Capto DEAE	1 × 4.7 mL	28926982
HiScreen Capto Q	1 × 4.7 mL	28926978
HiScreen Capto S	1 × 4.7 mL	28926979
ReadyToProcess Capto Q	2.5 L	28901723
	5 L	29146143
	10 L	28901724
	1 L	28951090
	20 L	28901725
ReadyToProcess Capto S	20 L	28901731
	2.5 L	28901729
	10 L	28901730
ReadyToProcess Capto S	1 L	28951093

Related literature

	Product code
Data files	
PreDictor 96-well filter plates and Assist software	28925839
PreDictor RoboColumn	28988634
HiScreen prepacked columns	28930581
ReadyToProcess columns	28915987
BPG columns	18111523
Chromaflow columns	18113892
AxiChrom columns	28929041
Application notes	
Methods for packing Capto S and Capto Q in production scale columns	28925932
Screening and optimization of loading conditions on Capto S	28407816
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