Capto SP ImpRes
Capto Q ImpRes

ION EXCHANGE CHROMATOGRAPHY

Capto™ SP ImpRes and Capto Q ImpRes are strong cation and strong anion exchangers for the high-throughput intermediate purification and polishing steps of a wide range of biomolecules. Both chromatography media (resins) are part of platform of high-resolution media based on the Capto product line.

By combining the high-flow characteristics of Capto media with a small particle size, Capto SP ImpRes and Capto Q ImpRes deliver excellent pressure-flow properties with excellent resolution. The ability to run at higher flow rates and higher bed heights increases productivity and flexibility in process design.

Key benefits of Capto SP ImpRes and Capto Q ImpRes include:

- High-resolution intermediate purification and polishing based on the well-established Capto platform with traditional ligands
- Flexible process design due to large operational window of flow rates and bed heights
- High-throughput purifications easy to optimize and scale up
- Higher manufacturing productivity enables improved process economy
- Security of supply and comprehensive regulatory support

Chromatography media characteristics

Ionic groups and base matrix

Capto SP ImpRes and Capto Q ImpRes are strong cation and strong anion exchangers, respectively. The ligand of Capto SP ImpRes is a sulfonate group while the ligand of Capto Q ImpRes is a quaternary amine group (Fig 2).

Fig 1. Capto SP ImpRes and Capto Q ImpRes extend the well-established Capto platform to include high-resolution media for late intermediate purification and polishing.

Capto ImpRes media are based on a high-flow agarose base matrix with good pressure/flow properties (Fig 3) and a small bead size (approx. 40 µm), which gives high resolution. These features, in combination with the well-established SP and Q ligands, make Capto SP ImpRes and Capto Q ImpRes an excellent choice for reliable intermediate purification and polishing in a commercial setting. Table 1 lists further characteristics of the media.

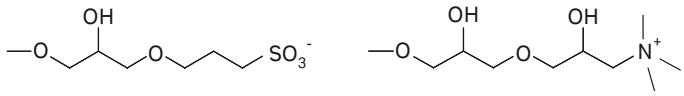


Fig 2. The strong ion exchange ligand groups of Capto SP ImpRes (left) and Capto Q ImpRes (right) are well established in large-scale purifications.

cytiva

Table 1. Main characteristics of Capto SP ImpRes and Capto Q ImpRes

	Capto SP ImpRes	Capto Q ImpRes
Matrix	High-flow agarose	High-flow agarose
Functional group	$-CH_2CH_2CH_2SO_3^-$	$-CH_2N^+(CH_3)_3$
Total ionic capacity	0.13 to 0.16 mmol (H^+)/mL medium	0.15 to 0.18 mmol (Cl^-)/mL medium
Average particle size (d_{50v})*	40 µm	40 µm
Flow velocity†	At least 220 cm/h in a 1 m diameter column with bed height 20 cm at 20°C; measured using process buffers with the same viscosity as water at < 3 bar (0.3 MPa, 43.5 psi)	
Binding capacity‡	> 70 mg lysozyme/mL medium, > 95 mg BSA/mL medium	> 55 mg BSA/mL medium, > 48 mg β-lactoglobulin/mL medium
Operating pH stability§		
Cleaning	3 to 14	2 to 14
Working	4 to 12	2 to 12
Working temperature	4°C to 30°C	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M sodium hydroxide¶, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, and 70% ethanol	
Avoid	Oxidizing agents, cationic detergents	Oxidizing agents, anionic detergents
Storage	20% ethanol, 0.2 M sodium acetate at 4°C to 30°C	20% ethanol at 4°C to 30°C

* d_{50v} is the average particle size of the cumulative volume distribution.
† Flow velocity stated in the Table is dependent on the column used, see Table 3.
‡ Dynamic binding capacity at 10% breakthrough measured at a residence time of 4 min (150 cm/h) in a Tricorn™ 5/100 column with 10 cm bed height. Buffer conditions used were for Capto SP ImpRes: 20 mM sodium phosphate, pH 7.2 (lysozyme) and 50 mM Tris, pH 8.0 (BSA); and for Capto Q ImpRes: 50 mM sodium acetate, pH 4.75 (BSA and β-lactoglobulin)
§ Cleaning pH: pH interval where the medium can be subjected to cleaning or sanitization-in-place without significant change of function. Working pH: pH interval where the medium can be operated without significant change of function.
¶ No significant change in ionic capacity and carbon content after 1 week storage in 1 M NaOH at 40°C.

High-throughput processing improves productivity

The rigidity of Capto ImpRes media permits high flow velocities; at least 220 cm/h for Capto SP ImpRes and Capto Q ImpRes in a 1 m diameter column with 20 cm bed height. Backpressure is nevertheless below 3 bar (0.3 MPa, 43.5 psi). Note that flow velocities are column dependent (Table 3).

This pressure/flow performance is improved compared to that obtained with previous generations of agarose polishing media while still giving the same, high resolution. Figure 3 compares Capto ImpRes with Sepharose™ High Performance in a representative large-scale situation with an AxiChrom™ 300 column that gives negligible wall support. Although bead sizes are similar, the pressure/flow properties of Capto ImpRes are significantly improved as a result of the greater mechanical stability of its high-flow base matrix.

The resulting high throughput increases processing productivity. Large volumes can be processed in one working shift, for example. Table 2 summarizes results that compare productivity data for Capto SP ImpRes and SP Sepharose High Performance during the second step of a monoclonal antibody purification. Calculations are based on experimental data at small scale. A simulation was made for processing 10 kg of MAb at a concentration of 15 g/L. Bed height was predefined and the column diameter was adjusted to get the column volume needed. Running conditions were the same for both media and the dynamic binding capacity was determined for each residence time. Column volume was calculated from the dynamic binding capacity (load 75% of Q_{B10}).

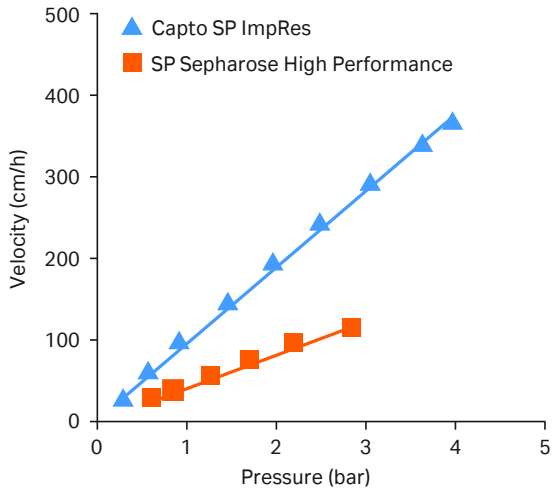


Fig 3. The pressure/flow properties of Capto ImpRes are superior to Sepharose High Performance due to the improved mechanical stability of the base matrix. Running conditions: AxiChrom 300 column, 20 cm bed height with water at 20ºC.

Table 2. Productivity simulation results from a MAb purification step confirm the higher productivity of Capto SP ImpRes

Chromatography medium	Flow velocity (cm/h)	Residence time (min)	Bed height (cm)	Time (h:min)	Productivity (kg/h)	Productivity (g/h/L medium)
Capto SP ImpRes	300	4	20	3:16	2.57	18.6
SP Sepharose High Performance	100*	12*	20	9:19	0.92	8.1

*The flow velocity and residence time had to be adjusted for SP Sepharose High Performance on account of the pressure/flow characteristics of the medium (Fig 4). The flow rate had to be decreased at 20 cm bed height.

The performance was similar for both media regarding host cell protein removal (2 to 2.5-fold reduction) and yield (approx. 85%). However, productivity was higher for Capto SP ImpRes (18.6 kg/h/L compared to 8.1 kg/h/L for the Sepharose SP High Performance example). Similarly, operating time was much shorter for Capto SP ImpRes. In addition, the more rigid Capto SP ImpRes medium allows much greater flexibility in choosing column sizes and operating conditions (Fig 4).

Wide window of operation

Properties of Capto media, such as matrix rigidity, allow a wide working range of flow velocities, bed heights, and sample viscosities. High flow velocities increase volume throughput and reduce process time, higher bed heights mean smaller diameters and a reduced equipment footprint.

The freedom available in process design for a given chromatography medium can be illustrated as its "window of operation". Figure 4A shows the relationship between column bed height and operating flow velocity for two different ion exchangers based on Capto ImpRes and Sepharose High Performance matrices. Both media are composed of small beads (40 µm vs 34 µm) and are used for the intermediate purification/polishing step in large-scale purification schemes. The size of the area below the pressure-limit curves represents the window of operation, that is, the available operating range for the respective medium. As Figure 4A shows, this is significantly greater for Capto ImpRes than for Sepharose High Performance.

The higher mechanical stability of Capto ImpRes compared with Sepharose High Performance enables the use of higher flow velocities in combination with more practical and cost-effective, smaller diameter columns.

The pressure limits for Capto ImpRes and Sepharose High Performance media in Figure 4A are based on a process diameter column and calculated for 20 cm bed height and maximum operating flow velocities of 220 and 110 cm/h, respectively. At these flow velocities, the pressure is equal to or less than 3 bar (0.3 MPa, 43.5 psi) for Capto ImpRes and 2 bar (0.2 MPa, 29 psi) for Sepharose High Performance, which is the highest recommended operating pressure for this medium at this scale. An operating pressure of 3 bar corresponds to the maximum pressure for many low-pressure systems; Capto ImpRes as such can normally be run at the maximum pressure rating of low- and medium-pressure columns.

Figure 4B shows the pressure-limit curves and windows of operation for two ion exchangers based on Capto ImpRes and Capto matrices. The larger bead size (90 µm) of the Capto medium allows greater flow velocities and operating pressures over a wide range of bed heights and is more suited as a capture medium during the first step in purification schemes.

Note that the maximum flow velocity of Capto ImpRes is dependent on the column used. For recommended columns, see Table 3. For details of maximum flow velocities possible with different columns, see Table 3.

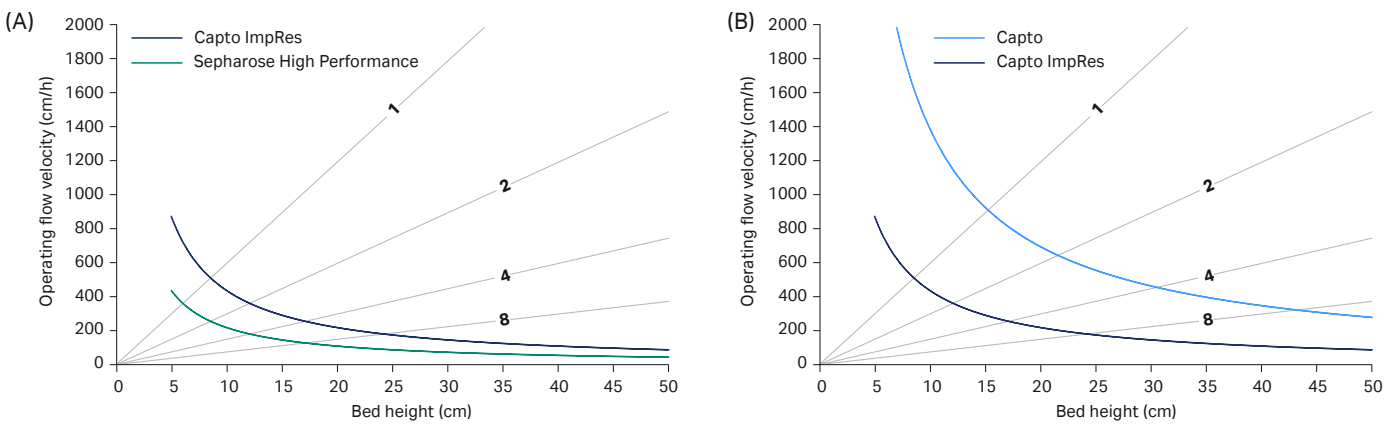


Fig 4. The window of operation (area below the curves) of (A) Capto ImpRes compared with Sepharose High Performance and (B) Capto ImpRes compared with Capto medium. Data correspond to a process diameter column at 20°C and viscosity equivalent to water. Gray contours give the residence time in the column in minutes.

High resolution for late-stage purification and polishing

The charged groups of the SP and Q ligands used in Capto ImpRes media are identical to the SP and Q charged groups used in other Cytiva ion exchange media. However, differences in base matrix, ligand density, and surface extenders can lead to differences in resolution. Figure 5 illustrates this for four 'Q-type' ion exchangers – Capto Q ImpRes, Q Sepharose High Performance, Q Sepharose Fast Flow and Capto Q – in HiScreen™ prepacked columns. As can be seen by the sharper peaks, Capto Q ImpRes and Q Sepharose High Performance both deliver improved resolution. However, the high-flow properties of Capto Q ImpRes make it the first-choice for large-scale use.

BioProcess chromatography media

Capto SP ImpRes and Capto Q ImpRes are BioProcess™ media, which is a family of purification media widely used by biopharmaceutical manufacturers. Support for these products includes validated manufacturing methods, secure long-term media supply, safe and easy handling, and Regulatory Support Files (RSF) to assist process validation and submissions to regulatory authorities. In addition, the Fast Trak Training & Education team provide high-level, hands-on training for all key aspects of bioprocess development and manufacturing.

Columns:	HiScreen Capto Q ImpRes, HiScreen Q HP, HiScreen Capto Q, HiScreen Q FF
Column volumes:	4.7 mL
Sample:	5 mL apo-transferrin (0.3 mg/mL), α-lactoalbumin (0.4 mg/mL), soybean trypsin inhibitor (0.6 mg/mL) in start buffer
Start buffer:	50 mM Tris, pH 7.4
Elution buffer:	50 mM Tris, 0.5 M NaCl, pH 7.4
Flow rate:	HiScreen Q HP: 1.2 mL/min (150 cm/h), HiScreen Capto Q ImpRes and HiScreen Q FF: 2.3 mL/min (300 cm/h), HiScreen Capto Q: 3.1 mL/min (400 cm/h)
Gradient:	0% to 100% elution buffer in 20 CV
Residence time:	HiScreen Q HP: 4 min, HiScreen Capto Q ImpRes and HiScreen Q FF: 2 min, HiScreen Capto Q: 1.5 min
System:	ÄKTA™ avant 25

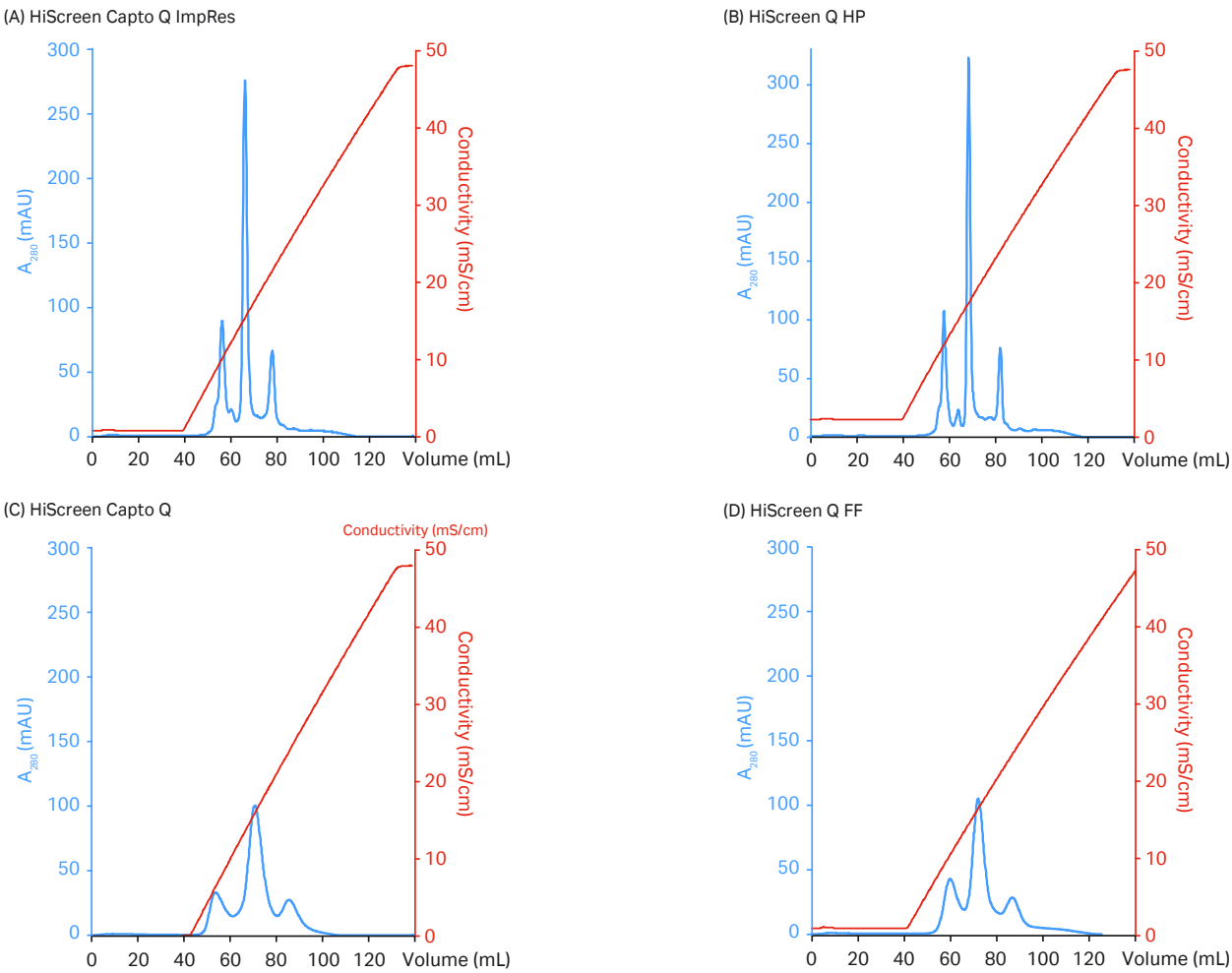


Fig 5. Chromatograms from resolution comparisons on four ion exchange media with the same Q charged group ligand. Peaks (left to right) are apo-transferrin, α-lactoalbumin, and soybean trypsin inhibitor. The smaller bead size gives (A) Capto Q ImpRes and (B) Q Sepharose High Performance improved resolution compared with (C) Capto Q and (D) Q Sepharose Fast Flow. Capto Q ImpRes is an excellent choice for large-scale intermediate purification or polishing due to its high flow properties.

Operation

Equipment

Capto SP ImpRes and Capto Q ImpRes can be used with most modern chromatography equipment from laboratory to production scale. Due to the higher rigidity of both media, packing procedures differ slightly compared to Sepharose High Performance media (for details of packing laboratory-scale columns, see the appropriate Instructions). There are also differences in packing procedures between pilot- and production-scale columns. The maximum flow rate is slightly reduced in Chromaflow™ columns (Table 3).

Table 3 lists suitable empty columns from Cytiva. Small-format columns prepacked with Capto SP and Q ImpRes are summarized in Tables 4 and 5.

Table 3. Cytiva column families for packing Capto SP ImpRes and Capto Q ImpRes

Column family	Inner diameter (mm)
Laboratory scale:	
Tricorn	5, 10
HiScale™	16, 26
Pilot and production scale:	
AxiChrom*	50 to 1000
BPG™†	100 to 300
Chromaflow‡	400 to 600

* Maximum bed height for AxiChrom 1000 is 20 cm.
† Note that the pressure rating of BPG 450 column is too low for Capto ImpRes media.
‡ Chromaflow columns with inner diameters > 600 mm are not recommended for packing with Capto ImpRes media. Maximum flow velocity for Chromaflow 600 is approximately 150 cm/h at 20 cm bed height and 20°C using process buffers with the same viscosity as water.

Small-scale formats give fast screening and method development

Using small-scale formats to screen for the most suitable chromatography media and/or process conditions in the early stages of process development saves both time and sample.

Capto SP ImpRes and Capto Q ImpRes are available in 96-well PreDictor™ plates, which support high-throughput process development (HTPD) by allowing parallel screening of chromatographic conditions such as pH and conductivity. The media are also available in PreDictor RoboColumn™ format. These miniaturized columns are prepacked with BioProcess media for HTPD using robotic liquid handling workstations.

Both media are also available in the small prepacked column formats HiTrap™ 1 mL and 5 mL and HiScreen (4.7 mL). Together with a chromatography system such as ÄKTA avant, prepacked HiTrap and HiScreen are convenient to use when developing an efficient and robust separation method. Further development and optimization using Tricorn or HiScale columns then permits straightforward scale-up. Basic characteristics of HiTrap and HiScreen prepacked columns are summarized in Tables 4 and 5, respectively.

Table 4. Main characteristics of HiTrap columns prepacked with Capto Q ImpRes or Capto SP ImpRes

Column volume	1 mL and 5 mL
Column dimensions	0.7 × 2.5 cm (HiTrap 1 mL), 1.6 × 2.5 cm (HiTrap 5 mL)
Maximum flow rates	4 mL/min (HiTrap 1 mL), 20 mL/min (HiTrap 5 mL)
Recommended flow rates	1 mL/min (HiTrap 1 mL), 5 mL/min (HiTrap 5 mL)
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)

Table 5. Main characteristics of HiScreen columns prepacked with Capto Q ImpRes or Capto SP ImpRes

Column volume	4.7 mL
Column dimensions	0.77 × 10 cm
Maximum flow rates	300 cm/h (2.3 mL/min)
Recommended flow rates	100 to 300 cm/h
Column hardware pressure limit	8 bar (0.8 MPa, 116 psi)

Fully scalable

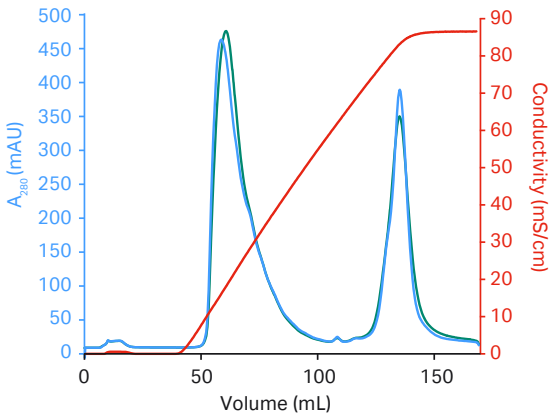
Scale-up is typically performed by keeping bed height and flow velocity constant while increasing column bed diameter and flow rate. However, since conditions are often optimized in small column volumes, parameters such as dynamic binding capacity can be optimized on shorter bed heights than those used at the final scale. Nevertheless, as long as the residence time is constant, the binding capacity for the target molecule remains the same. To utilize the full large-scale potential of Capto SP ImpRes and Capto Q ImpRes, we recommend bed heights of 20 to 40 cm (see example below). Note that maximum bed height in AxiChrom 1000 is 20 cm due to the high packing forces of the media (Table 3).

BSA and lactoferrin were separated on Capto SP ImpRes at three different scales and two different flow rates (150 cm/h and 300 cm/h). The columns used were two prepacked HiScreen Capto SP ImpRes columns connected in series (total 9.3 mL, 20 cm bed height), HiScale 16/20 (40 mL, 20 cm bed height) and AxiChrom 50 (398 mL, 20.3 cm bed height). Linear flow velocity was kept constant while sample volume was increased in proportion to the column volumes. Figure 6 shows the results.

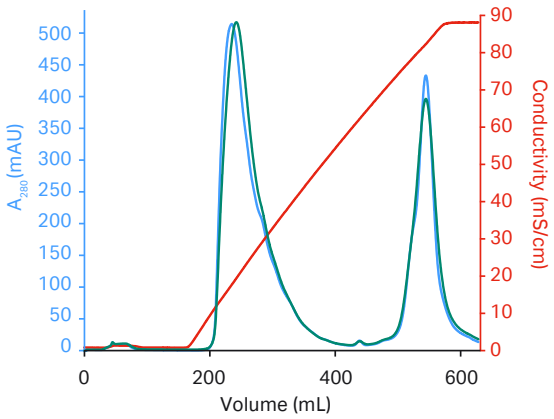
The three column sizes all gave comparable purifications. Chromatograms also show that resolution at the higher flow rate was almost identical to that of the lower flow rate. Chromatographic performance was thus maintained during scale-up and increased flow rate.

Columns:	2 × HiScreen Capto SP ImpRes (0.77 × 20 cm), 9.3 mL total; HiScale 16/20 packed with Capto SP ImpRes (1.6 × 20 cm), 40 mL; AxiChrom 50 packed with Capto SP ImpRes (5 × 20.3 cm), 398 mL
Sample:	7.5 mg/mL BSA and 2.5 mg/mL lactoferrin
Sample volume:	1 CV
Start buffer:	50 mM acetate, pH 5
Elution buffer:	50 mM acetate, 1 M NaCl, pH 5
Flow rate:	150 cm/h and 300 cm/h
Gradient:	0% to 100% elution buffer in 10 CV
Residence time:	4 or 8 min depending on flow rate
System:	ÄKTA avant 150

(A) Two HiScreen columns in series (9.3 mL)



(B) HiScale column (40 mL)



(C) AxiChrom 50 column (398 mL)

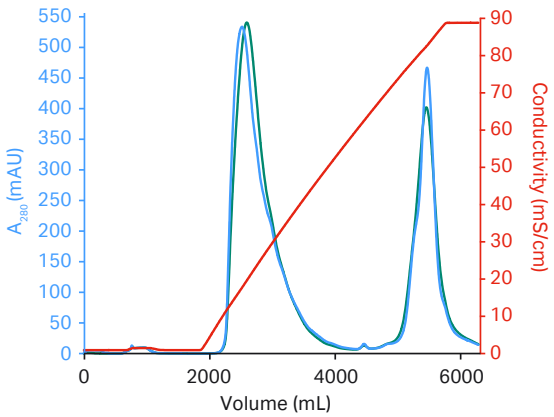


Fig 6. Separation of BSA and lactoferrin at three increasing scales (9.3, 40, and 398 mL columns) and two flow rates (150 cm/h [blue curves] and 300 cm/h [green curves]). Chromatographic performance was maintained during scale-up and at increased flow rate.

Cleaning and sanitization

Cleaning-in-place (CIP) is a cleaning procedure to remove 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 chromatography bed and helps maintain the capacity, flow properties, and general performance of the medium.

The frequency of CIP depends on the nature and the condition of the starting material, but one CIP cycle is generally recommended every one to five separation cycles. Furthermore, a specific CIP protocol should be designed for each process according to the type of contaminants present. Note that Capto SP ImpRes and Capto Q ImpRes both withstand standard CIP solutions such as 1 M NaOH, 2 M NaCl, and 70% ethanol, or combinations thereof.

Regular sanitization will hinder microbial growth and maintain a high level of hygiene in the packed column. A specific sanitization protocol should be developed according to the nature and condition of the starting material. Sanitization protocols based on 0.5 M to 1.0 M NaOH can be used for Capto SP ImpRes and Capto Q ImpRes.

Applications

The main challenge for late intermediate purification and polishing is often to selectively remove impurities similar to the target product, for example, aggregated forms and protein structural or sequence variants, as well as key contaminants.

The high-resolution of Capto SP ImpRes and Capto Q ImpRes, combined with their high-flow characteristics, offers an improved solution to this challenge for the production-scale purification and/or polishing of many classes of biomolecule.

Efficient removal of aggregates in a monoclonal antibody purification process

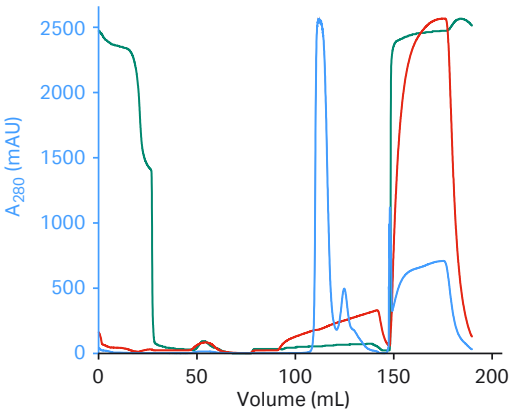
A sample of a monoclonal IgG antibody from an initial protein A capture step contained a high amount of covalent aggregated forms (approx. 11%). Capto SP ImpRes was used to remove these aggregates in the subsequent second-step purification.

Aggregate levels in the fractions were determined by gel filtration. Host cell protein (HCP) content was analyzed with ELISA and yield was determined by measuring absorbance at 280 nm. Two sample loads (10 g/L and 50 g/L) were run. Figure 7 shows the resulting chromatograms and Figure 8 the aggregate content as a function of yield.

Capto SP ImpRes successfully separated antibody aggregates from the monomeric form at both loads. At 10 g/L, aggregate content was reduced to less than 1% and monomer was 81%. For 50 g/L, the equivalent figures were less than 1% and 84%. High resolution was thus maintained at the higher load. At the same time, HCP content was reduced by a factor of approximately 9 to 15-fold, depending on pooling criteria.

Column:	0.66 cm i.d., 20 cm bed height (CV, 6.8 mL)
Medium:	Capto SP ImpRes
Sample:	Eluted IgG pool from protein A capture step
Sample load:	10 g/L and 50 g/L
Start buffer:	50 mM sodium acetate, pH 5.5
Elution buffer:	500 mM sodium acetate, pH 5.5
Flow rate:	150 cm/h
Linear gradient:	10% to 90% elution buffer in 15 CV
Residence time:	8 min
System:	ÄKTAexplorer 100

(A) Load 10 g/L



(B) Load 50 g/L

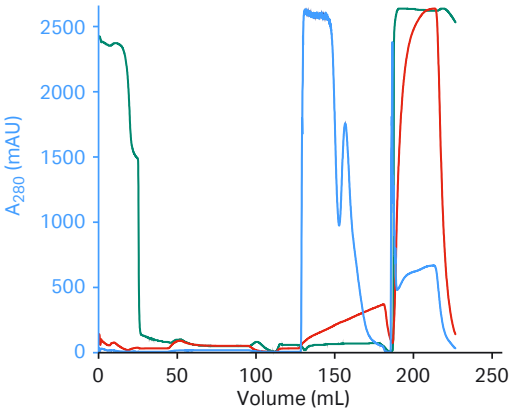


Fig 7. Separation of antibody aggregates from a monomeric form at (A) 10 g/L and (B) 50 g/L sample loads on Capto SP ImpRes. Absorbance (280 nm) shown in blue, conductivity in red, pH in green.

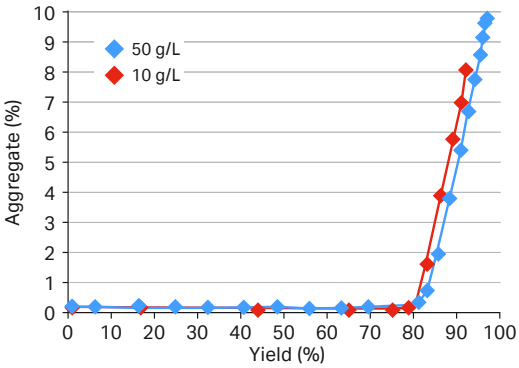


Fig 8. Aggregate content as a function of yield at two different sample loads.

Selective removal of contaminants in an insulin purification process

Capto SP ImpRes was used as an intermediate step in an insulin purification process to separate insulin from desthreonine-insulin, desamido-insulin, C-peptide, and other cleaving components that remain following initial purification. Desthreonine and desamido are insulin variants that only differ in one amino acid or less. The resulting chromatogram is shown in Figure 9.

Insulin and truncated insulin eluted in the first step. Insulin (first peak) was well resolved from truncated insulin impurities (middle peak). C-peptides eluted as a later peak. Pooled fractions were analyzed by reversed phase chromatography, which showed that insulin purity increased from 64% to 91% during this one step. The truncated insulin content was reduced from 11.5% to 2.8%.

Column:	Tricorn 5/50 CV 1 mL
Medium:	Capto SP ImpRes
Sample:	18 mg cleaved insulin*
Start buffer:	50 mM acetate, 47.5% ethanol, pH 4
Elution buffer:	50 mM acetate, 47.5% ethanol, 1 M NaCl, pH 4
Flow rate:	0.4 mL/min (120 cm/h)
Elution:	First step: 47.5% ethanol, 130 mM NaCl, pH 4 (10 CV) Second step: 47.5% ethanol, 1 M NaCl, pH 4 (5 CV)
Residence time:	2.5 min
System:	ÄKTA avant 25

** Kindly provided by Biomm S.A. (Brazil).*

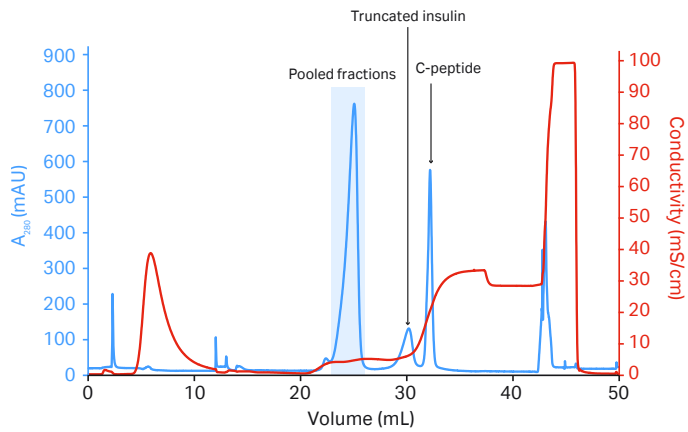


Fig 9. The intermediate step purification of insulin on Capto SP ImpRes removes contaminants such as desthreonine- and desamido-insulin.

Separation of intact and truncated forms of a recombinant protein

Capto SP ImpRes was used to separate a recombinant protein from a truncated form of the same protein, which is an exceptionally challenging task to solve.

Based on results from initial purifications run with gradient elution (not shown), a two-step gradient elution was optimized to separate the protein from its truncated form. Figure 10 shows this separation. Fractions were analyzed by SDS-PAGE (Fig 11) and analytical gel filtration (Fig 12).

Column:	HiScreen Capto SP ImpRes, 4.7 mL
Sample:	Recombinant protein purified on IgG Sepharose 4B; contains a truncated form of the protein
Sample load:	25 g/L
Start buffer:	25 mM citrate, pH 3.5
Elution buffer:	25 mM citrate, 500 mM NaCl, pH 3.5
Flow rate:	300 cm/h
Elution:	First step: 30% elution buffer in 7 CV Second step: 25 mM citrate, pH 6 in 5 CV
Residence time:	2 min
System:	ÄKTA avant 25

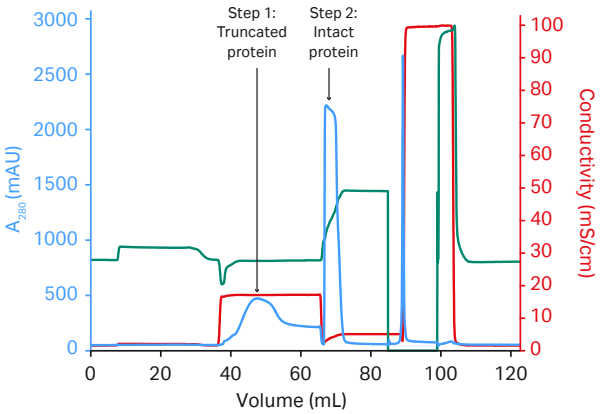
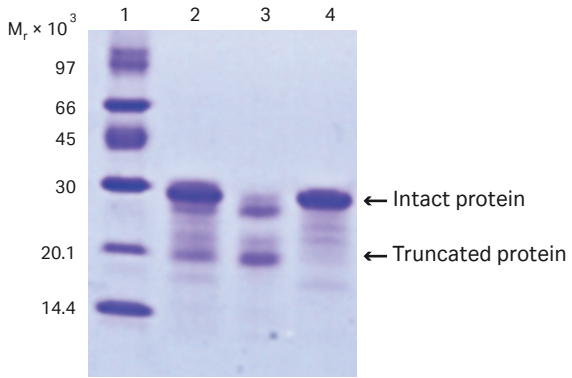


Fig 10. Purification of recombinant protein on HiScreen Capto SP ImpRes by two-step gradient elution. Peak 1 (Step 1) contains the truncated form of the protein. The intact protein elutes in the second peak (Step 2); pH is shown in green.



Lanes
1. Low Molecular Weight (LMW) marker
2. Starting material
3. Elution step 1
4. Elution step 2

Fig 11. SDS-PAGE of the eluted fractions under nonreducing conditions (PhastGel™ Homogenous 20), Coomassie™ stained.

Columns:	2 × Superdex™ 200 5/150 GL connected in series, total CV 6 mL
Sample volume:	10 µL
Mobile phase buffer:	PBS, pH 7.4
Flow rate:	0.35 mL/min
System:	ÄKTAexplorer 10

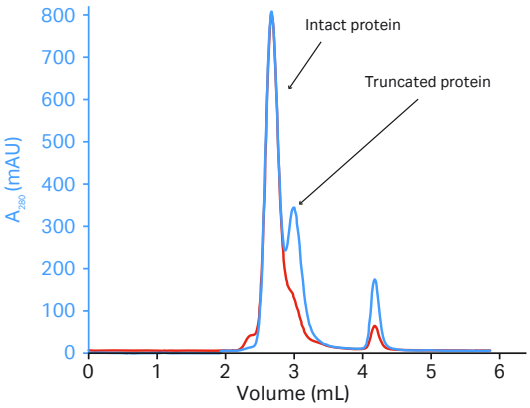


Fig 12. Gel filtration analysis of a recombinant protein and its truncated form before purification on HiScreen Capto SP ImpRes (blue) and after purification (red). Curves are normalized with respect to the protein peak in the start sample.

Both the SDS-PAGE and gel filtration analyses showed that the different forms of the protein were well separated on HiScreen Capto SP ImpRes; the first peak contained the truncated protein and the second peak the intact protein. The truncated protein was eluted by an increase in conductivity (first step) and the intact protein by an increase in pH without salt (second step). The decision to elute without salt was based on previous experience with precipitation at high salt concentration.

The yield for intact protein was 87% as calculated from gel filtration chromatograms.

Purification of green fluorescent protein (GFP) expressed in E. coli

The main goal of this study was to remove *E. coli* proteins remaining after an initial capture step. Like the previous example, this is a difficult separation to achieve successfully.

GFP was purified on Capto Q ImpRes using a salt gradient at pH 8. Figure 13 shows the result. The green absorbance curve is GFP-specific absorbance at 490 nm.

Fractions were analyzed by gel filtration and SDS-PAGE. Gel filtration showed that the Capto Q ImpRes step increased GFP purity to 93% (the GFP content in the start sample was 70%). The total yield for this purification step was 94%. Pooled fractions containing the purified GFP are run in Lane 3 in the SDS-PAGE gel shown in Figure 14.

Column:	Tricorn 5/100, CV 2 mL
Medium:	Capto Q ImpRes
Sample:	Partially purified recombinant GFP expressed in *E. coli*
Sample load:	20 g/L
Start buffer:	50 mM Tris, pH 8.0
Elution buffer:	50 mM Tris, 1 M NaCl, pH 8.0
Flow rate:	150 cm/h
Gradient elution:	0% to 100% elution buffer in 20 CV
Residence time:	4 min
System:	ÄKTA avant 25

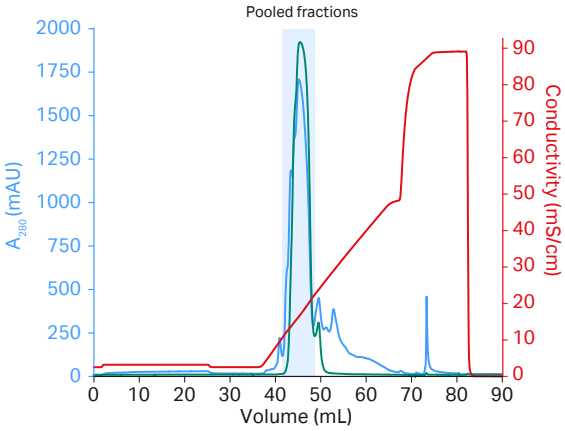
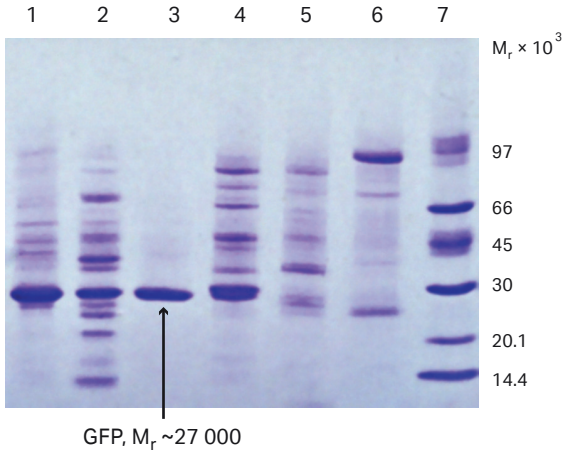


Fig 13. Purification of GFP on a Tricorn 5/100 column packed with Capto Q ImpRes. The marked pooled fractions contain the purified protein (see SDS-PAGE Lane 3 in Fig 14).



Lane

1.	Start sample
2.	Fraction before main peak at 41 mL
3.	Main peak containing GFP (marked pooled fractions in Fig 13)
4, 5, and 6.	Fractions after the main peak at 49 mL, 50 mL, and 52 mL
7.	Low Molecular Weight (LMW) marker

Fig 14. SDS-PAGE of fractions from the purification of GFP under nonreducing conditions (PhastGel Gradient 8), Coomassie stained. Pooled fractions were diluted 20-fold, start sample and other fractions were diluted 2-fold.

Storage

Store Capto SP ImpRes and Capto Q ImpRes media and prepacked columns at 4°C to 30°C in 20% ethanol. An alternative storage solution is 2% benzyl alcohol. For Capto SP ImpRes, always add 0.2 M sodium acetate irrespective of which of the above solutions is used.

Ordering information

Product	Quantity	Code number
Capto SP ImpRes	25 mL	17-5468-10
Capto SP ImpRes	100 mL	17-5468-02
Capto SP ImpRes	1 L	17-5468-03
Capto SP ImpRes	5 L	17-5468-04
Capto SP ImpRes	10 L	17-5468-05
Capto Q ImpRes	25 mL	17-5470-10
Capto Q ImpRes	100 mL	17-5470-02
Capto Q ImpRes	1 L	17-5470-03
Capto Q ImpRes	5 L	17-5470-04
Capto Q ImpRes	10 L	17-5470-05
HiTrap Capto SP ImpRes	5 × 1 mL	17-5468-51
HiTrap Capto SP ImpRes	5 × 5 mL	17-5468-55
HiScreen Capto SP ImpRes	1 × 4.7 mL	17-5468-15
HiTrap Capto Q ImpRes	5 × 1 mL	17-5470-51
HiTrap Capto Q ImpRes	5 × 5 mL	17-5470-55
HiScreen Capto Q ImpRes	1 × 4.7 mL	17-5470-15
PreDictor Capto SP ImpRes, 6 µL	4 × 96-well filter plates	17-5468-16
PreDictor Capto SP ImpRes, 20 µL	4 × 96-well filter plates	17-5468-17
PreDictor Capto Q ImpRes, 6 µL	4 × 96-well filter plates	17-5470-16
PreDictor Capto Q ImpRes, 20 µL	4 × 96-well filter plates	17-5470-17
PreDictor RoboColumn Capto Q ImpRes	200 µL	28-9969-18
PreDictor RoboColumn Capto Q ImpRes	600 µL	28-9973-91
PreDictor RoboColumn Capto SP ImpRes	200 µL	28-9974-49
PreDictor RoboColumn Capto SP ImpRes	600 µL	28-9974-50

Accessories	Quantity	Code number
1/16″ male/Luer female*	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16″ female/M6 male	6	18-1112-57
Union M6 female/1/16″ male	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep™, 1/16″ male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16″†	5	11-0004-64
Fingertight stop plug, 1/16″‡	5	11-0003-55

* One connector included in each HiTrap package.
† Two, five, or seven stop plugs female included in HiTrap packages depending on products.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature

Data Files	Code number
PreDictor 96-well filter plates and Assist software	28-9258-39
HiScreen prepacked columns	28-9305-81
AxiChrom columns	28-9290-41
BPG columns	18-1115-23
Chromaflow columns	18-1138-92
Application note	
Methods for packing Capto Q ImpRes and Capto SP ImpRes in production-scale columns	29-0306-98
Handbooks	
Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11-0004-21
High-throughput Process Development with PreDictor plates: Principles and Methods	28-9403-58
Selection guides	
Ion exchange columns and media	18-1127-31
Prepacked chromatography columns for ÄKTA systems	28-9317-78

cytiva.com/bioprocess

CY12674-03Jul20-DF

cytiva