Capto Q XP

ION EXCHANGE CHROMATOGRAPHY

Capto™ Q XP is a strong anion exchange resin designed for the purification of large biomolecules such as immunoglobulins (e.g., IgG, IgM, IgA) from plasma (Fig 1). Capto Q XP is part of Cytiva's Custom Designed Media program.

Key benefits of Capto Q XP include:

- Efficient industrial-scale capture and intermediate purification of large biomolecules 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 to improve manufacturing productivity and process economy
- Excellent chemical stability

Fig 1. Capto Q XP extends the well-established Capto platform to include resin for intermediate purification and polishing of large biomolecules.

Resin characteristics

Designed to separate large biomolecules efficiently

Capto Q XP is based on a high-flow agarose base matrix with good pressure-flow properties and wide pore openings to give high available surface area for adsorption of large biomolecules. The Q ligand of Capto Q XP is a quaternary amine group (Fig 2).

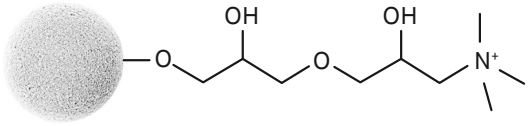

Fig 2. The strong anion exchange ligand of Capto Q XP, comprising a quaternary amine group, is well established in large-scale purifications.

The features of the base matrix, in combination with the conventional Q ligand, make Capto Q XP an excellent choice for high productivity capture and intermediate purification of large biomolecules. The basic characteristics of the resin are listed in Table 1.

BioProcess chromatography resins

Capto Q XP is part of the BioProcess™ resins, a family of purification resins widely used by biopharmaceutical manufacturers. Support for these products includes secure long-term resin supply as well as secure and easy handling. Regulatory support files (RSFs) are available for these products, to assist in process validation and submissions to regulatory authorities. In addition, the Fast Trak training and education team provides high-level, hands-on training for all key aspects of bioprocess development and manufacturing.

Table 1. Main characteristics of Capto Q XP

Matrix	High-flow agarose
Particle size*	75 µm (d_{50v})
Functional group	$-CH_2N^+(CH_3)_3$
Total ionic capacity	95–125 µmol (Cl^-)/mL resin
Flow velocity†	Up to 300 cm/h in a 1 m diameter column with a bed height of 20 cm at 20°C; measured using process buffers with the same viscosity as water at 300 kPa.
pH stability (operational)‡	pH 2–12
CIP stability (short-term)¶	pH 2–14
Chemical stability	Stable in commonly used buffer solutions: 1 M sodium hydroxide, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol, 30% isopropanol
Shelf life	Five years
Storage conditions	20% ethanol at 4°C to 30°C

* d_{50v} is the median particle size for the cumulative volume distribution.
† Flow velocities are dependent on the column used.
‡ pH interval where the resin can be operated without significant change in function.
¶ pH interval where the resin can be subjected to cleaning in place (CIP) without significant change in function.

Application: purification of intravenous IgG (IVIG)

One of the challenges in chromatographic purification of IVIG is to remove large molecular weight impurities such as IgM and IgA. When screening chromatography resins, dynamic binding capacity and pressure-flow properties are key attributes to be considered. Designed with strong anion exchange groups attached to a high-flow agarose base matrix with wide pore openings, Capto Q XP is optimized for high binding of large molecular weight proteins.

Process design

The IVIG purification process is outlined in Figure 3. After traditional IVIG precipitation, fraction (I+) II+III was resuspended in low pH and treated with caprylic acid to remove lipids and other impurities. IVIG in the resulting supernatant was purified using two anion exchange (AIEX) chromatography steps in flow-through mode. In the first AIEX step, standard Capto Q resin was used and found to be effective in removing all traces of albumin from the fraction (I+) II+III supernatant. In addition, approximately 50% of IgA was removed in this step. In the second AIEX step, Capto Q XP was used for final polishing of IVIG. In this step, both IgM and residual IgA were effectively removed from IVIG in the flowthrough.

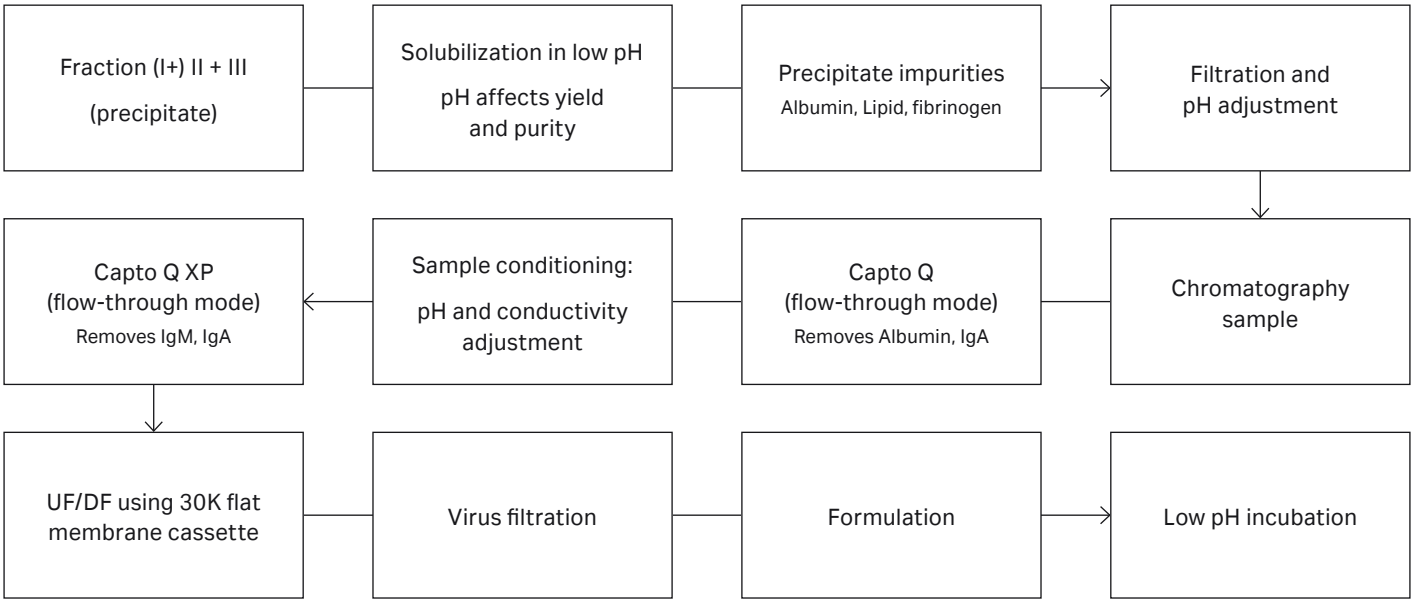

Fig 3. IVIG purification process design, from fraction (I+) II+III to final product. UF/DF = ultrafiltration/diafiltration.

Dynamic binding capacity for IgA and IgM

The flowthrough from the Capto Q step only contained IgG, IgA, and IgM. The pH in the flowthrough was adjusted to pH 6.0 using NaOH and conductivity to 0.8 mS/cm using water for injection (WFI). Commonly, the concentrations of IgG, IgA, and IgM are 3–6 g/L, 0.1–0.3 g/L, and 0.1–0.3 g/L, respectively. In the Capto Q XP step, IgA and IgM bound to the column and IgG remained in the flowthrough. IgM content was the key factor and loading was stopped when IgM was detectable in the flowthrough. To maximize capacity, a flow velocity of 75 cm/h and a bed height of 20 cm in a XK16/40 column were used to give a long residence time (16 min). Using the ÄKTA™ avant chromatography system, collected fractions could be kept cool (6°C) in the fraction collector until further use. Dynamic binding capacity of Capto Q XP for IgA and IgM is shown in Figure 4.

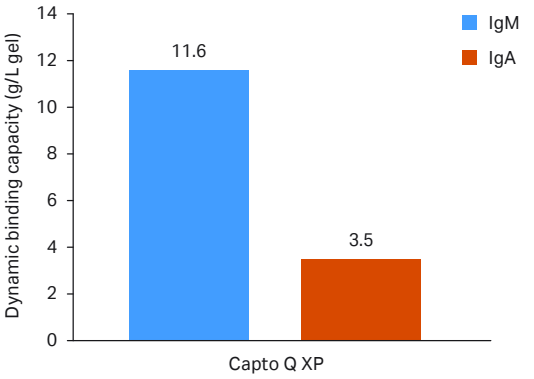

Fig 4. Dynamic binding capacity of Capto Q XP for IgA and IgM.

IVIG production at industry scale

In addition to resin capacity, also the operation flow rate should be taken into consideration in IVIG production at industry scale. Productivity is defined as purified protein per liter resin per hour (g protein/L resin/h). With a high capacity and good pressure-flow properties, Capto Q XP supports high productivity (Fig 5).

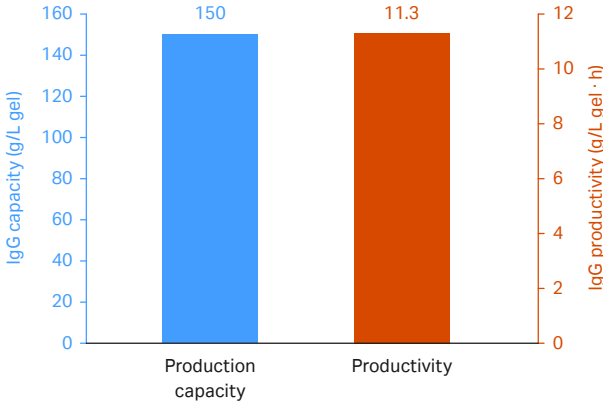

Fig 5. Production capacity and productivity using Capto Q XP.

Results

The IVIG-containing flowthrough was concentrated, diafiltered, and formulated to give a 5% final product. Release test results from a 500 L pilot-scale production of IVIG from plasma are listed in Table 2. As shown, albumin, IgA, and IgM content in the final product were 1.8 mg/L, 11.2 mg/L and 15.4 mg/L, respectively.

Table 2. Evaluation of the final IVIG product

Test	Target
Electrophoresis cellulose acetate (%)	99.9
HPLC analysis: monomer and dimer (%)	99.8
IgM (mg/L)	15.4
IgA (mg/L)	11.2
Albumin (mg/L)	1.8

Besides the high product quality, the process yield was also shown to be high. The pretreatment yield was more than 75%. The yield of the Capto Q step was more than 98%, and for the Capto Q XP step, yield was more than 92%. The overall yield for the process was more than 65%: 6.5 g IgG/L recovered from 10 g IgG/L in starting material. The yield and purity of the final IVIG product is significantly affected by sample pH. In the pilot run, a pH of 6.0 was shown to give both high purity and yield.

Stability

The ligand is immobilized to the agarose base matrix via stable bonds that ensure high chemical stability and low leakage. Figure 6 shows the stability of Capto Q XP after storage in different solutions of various pH at 40°C for one week. Both carbon and nitrogen leakage were shown to be low in the pH range 2 to 12. At pH > 12, carbon and nitrogen are released, which indicates hydrolysis of the ligand.

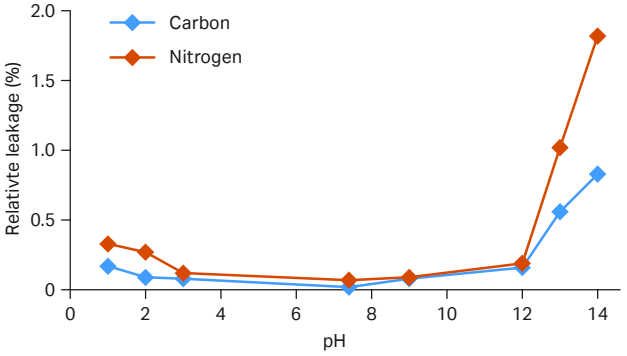

Fig 6. Stability of Capto Q XP at different pH values.

Cleaning in place (CIP) and sanitization in place (SIP)

CIP is a cleaning procedure to remove contaminants such as lipids, precipitates, or denatured proteins that might 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 resin.

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 Q XP withstands standard CIP solutions such as 1 M NaOH, 2 M NaCl, and 70% ethanol, or combinations thereof.

Regular SIP will prevent 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 to 1.0 M NaOH can be used for Capto Q XP.

Storage

The recommended storage conditions are 20% ethanol at 4°C to 30°C. Capto Q XP is supplied as a suspension in 20% ethanol.

Ordering information

Product	Quantity	Product code
Capto Q XP	25 mL	17547301
Capto Q XP	100 mL	17547302
Capto Q XP	1 L	17547303
Capto Q XP	5 L	17547304
HiTrap™ Capto Q XP	5 × 1 mL	17547311
HiTrap Capto Q XP	1 × 5 mL	17547312

Related literature	Product code
Handbook: Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	18000421

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