Capto HiRes ion exchange chromatography columns

ION EXCHANGE CHROMATOGRAPHY

Capto™ HiRes Q and Capto HiRes S are prepacked columns for high-resolution purification of proteins, peptides, polynucleotides, and other biomolecules by ion exchange chromatography, IEX (Fig 1).

The prepacked columns effectively separate biomolecules with small differences in net surface charge and are therefore well-suited to preparation of highly pure proteins in, for example, structural studies and protein analysis.

The columns offer the following benefits:

- High ion exchange chromatography resolution in protein and nucleic acid purification or analysis
- Excellent reproducibility and durability
- Versatility in a wide range of pH, ionic strengths, and gradient shape, to further exploit the charge characteristics of amino acids and other components on the surface of the biomolecule
- High chemical and pH stabilities
- Delivering similar or improved resolution and similar protein elution as MonoBeads (Mono Q™ and Mono S™) columns, which means smooth transition from one column protocol to the other

Ion exchange chromatography

Ion exchange chromatography separates molecules based on differences in their net surface charge. Molecules vary in their charge properties and will exhibit different degrees of interaction with a charged chromatography resin according to differences in their overall charge, charge density, and surface charge distribution. Charged molecules bind to the resin at specific ionic strengths and are then eluted with a salt or pH gradient.

Fig 1. Capto HiRes columns provide high-resolution purification of proteins, peptides, polynucleotides, and other biomolecules by ion exchange chromatography.

Ion exchange chromatography is one of the most frequently used and versatile methods for purification of biological substances, even proteins and peptides with small differences in charge can be separated. Furthermore, binding and elution conditions are easy to optimize, resulting in fast, high-resolution separations of biomolecules.

Chromatography resin characteristics

The chromatography resins in Capto HiRes Q and Capto HiRes S columns are based on a high-flow agarose base matrix with a small bead size of 9 µm. Capto HiRes Q and Capto HiRes S are strong anion and cation exchangers, respectively with ion exchange properties that remain constant during chromatography within the recommended pH range of 2 to 12.

Table 1 lists further characteristics of the resins.

Table 1. Capto HiRes Q and Capto HiRes S ion exchange chromatography resins

Properties	Capto HiRes Q	Capto HiRes S
Type of ion exchanger	Strong anion exchanger (quaternary ammonium)	Strong cation exchanger (methyl sulfonate)
Matrix	Highly cross-linked agarose, spherical	Highly cross-linked agarose, spherical
Particle size, d_{50v}[1]	~ 9 µm	~ 9 µm
Charged group	$-CH_2-N+(CH_3)_3$	CH_2-SO_3-
Ionic capacity/mL packed resin	~ 0.23 mmol Cl^-/mL resin	~ 0.12 mmol H^+/mL resin
Chemical stability (daily use)	• All commonly used aqueous buffers, pH 2–12 • Urea, up to 8 M • Acetonitrile, up to 30% in aqueous buffers • Nonionic detergents • Cationic detergents (for Capto HiRes Q) • Anionic detergents (for Capto HiRes S)	
Chemical stability (cleaning)	• Acetonitrile, up to 30% • Sodium hydroxide, up to 1 M • Ethanol, up to 70% • Methanol, up to 70% • Acetic acid, up to 1 M • Isopropanol, up to 30% • Hydrochloric acid up to 0.1 M • Guanidine hydrochloride, up to 6 M	
pH stability range	• Operational[2]: 2–12 • Cleaning in place (CIP)[3]: 1–14	
Temperature range	• Operating: 4°C to 40°C • Storage: 4°C to 30°C	

[1] Median particle size of the cumulative volume distribution.

[2] pH range where resin can be operated without significant change in function.

[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Chromatography column characteristics

Capto HiRes Q and Capto HiRes S resins are prepacked in high-performance Tricorn™ glass columns of two different sizes (Table 2). The columns have fittings for simple connection to ÄKTA™ chromatography systems or equivalent high-performance systems. All column parts are biocompatible.

Table 2. Characteristics of prepacked Capto HiRes Q and Capto HiRes S columns

	5/50 column	10/100 column
Bed dimensions	5 × 50 mm	10 × 100 mm
Column volume	1 mL	8 mL
Average sample loading capacity[1]	50 mg	400 mg
Flow rate[2]	Recommended: 0.5 to 2.0 mL/min	Recommended: 0.5 to 2.0 mL/min
	Maximum: 2.0 mL/min	Maximum: 3.0 mL/min
Maximum pressure over packed bed	4.0 MPa, (40 bar, 580 psi)	

[1] Sample dependent. For highest resolution, do not load more than 20% of the loading capacity.

[2] Recommended flow rates at room temperature for solutions with viscosity equivalent to water. Decrease the flow rate for solvents with higher viscosity such as 20% ethanol or at lower temperature.

High-resolution separation

In many research areas, for example in structural biology using X-ray crystallography or cryo-electron microscopy (cryo-EM), obtaining homogeneous size and charge of biomolecules is crucial for the elucidation of their structures. High-resolution separation of samples based on their charge properties is essential to secure sample charge homogeneity and success of the study.

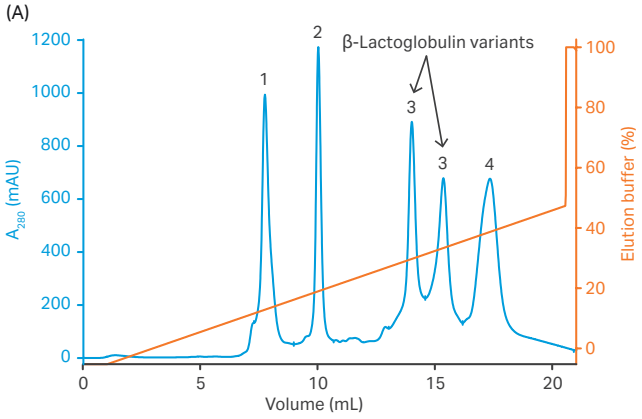
Examples of the high-resolution separations of Capto HiRes for two protein mixes are shown on a 1 mL anion exchange column and an 8 mL cation exchange column (Fig 2).

The first example is of Capto HiRes Q in a 1 mL 5/50 column. The four proteins in the mix are clearly baseline separated on the column. Also, the two variants of β-lactoglobulin are well separated, showing that the column can separate molecules that are very close in charge.

The second example is of Capto HiRes S in an 8 mL 10/100 column. The proteins also have a good baseline separation in the 10/100 column.

Column:	Capto HiRes Q 5/50
Sample:	1. Apotransferrin 2. α-Lactalbumin 3. β-Lactoglobulin 4. Amyloglucosidase
Sample volume:	500 µL
Sample load:	2.5 mg
Binding buffer:	20 mM Tris-HCl, pH 8.0
Elution buffer:	20 mM Tris-HCl, 1 M NaCl, pH 8.0
Gradient:	0% to 50% elution buffer in 20 CV
Flow rate:	1.0 mL/min (room temperature)

(A)



Column:	Capto HiRes S 10/100
Sample:	1. Ribonuclease A 2. α-Chymotrypsinogen A 3. Cytochrome C 4. Lysozyme
Sample volume:	800 µL
Sample load:	4.0 mg
Binding buffer:	20 mM phosphate, pH 6.8
Elution buffer:	500 mM phosphate, pH 6.8
Gradient:	0% to 50% elution buffer in 20 CV
Flow rate:	2.0 mL/min (room temperature)

(B)

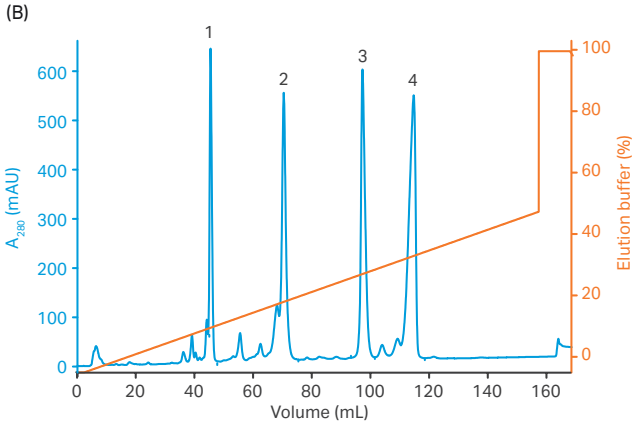


Fig 2. Capto HiRes columns can separate proteins that differ by only a few amino acids. (A) Separation of proteins on an anion exchange Capto HiRes Q 5/50 and (B) on a cation exchange Capto HiRes S 10/100.

Capto HiRes columns are comparable to MonoBeads columns

MonoBeads (Mono Q and Mono S) IEX columns have been the chromatography columns of choice since 1982 for high-resolution IEX when separations of closely charged biomolecules are needed.

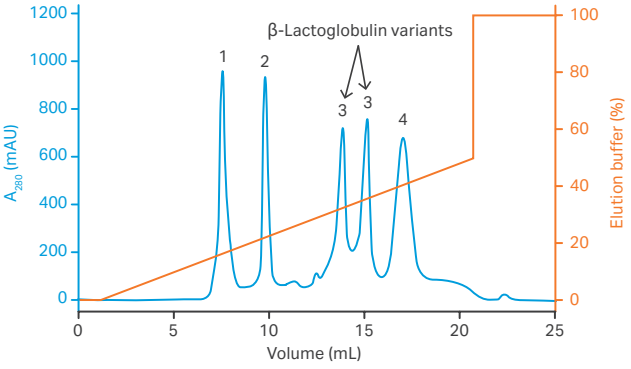
A separation that worked on a Mono Q or Mono S column may be performed on a Capto HiRes Q or Capto HiRes S column with little modification or optimization. Similar resin selectivity and slightly improved resolution can be expected with the Capto HiRes columns while using the same experimental conditions. The similar selectivity of the two columns ensures a smooth transition even for quality control (QC) applications.

Comparison of Mono Q 5/50 with Capto HiRes Q 5/50

Figure 3 shows the comparison of separations of protein mixtures on Mono Q 5/50 vs Capto HiRes Q 5/50 under identical experimental conditions. The elution profiles are very similar for these columns, with slightly higher resolution for Capto HiRes Q.

Column:	Mono Q 5/50 and Capto HiRes Q 5/50
Sample:	1. Apotransferrin 2. α-Lactalbumin 3. β-Lactoglobulin 4. Amyloglucosidase
Sample volume:	500 µL
Sample load:	2.5 mg
Binding buffer:	20 mM Tris-HCl, pH 8.0
Elution buffer:	20 mM Tris-HCl, 1 M NaCl, pH 8.0
Gradient:	0% to 50% elution buffer in 20 CV
Flow rate:	1.0 mL/min

(A)



(B)

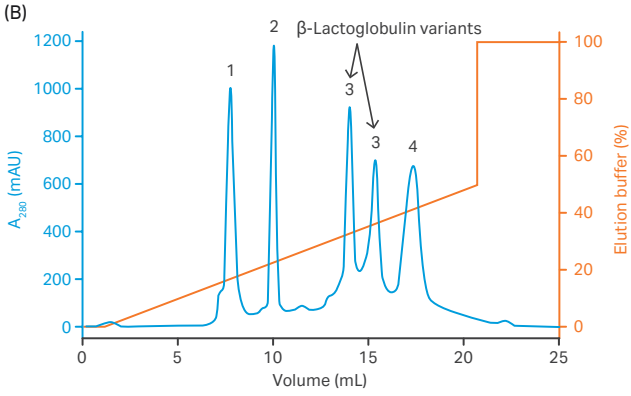


Fig 3. Separation of a sample consisting of four proteins on (A) Mono Q 5/50 and (B) Capto HiRes Q 5/50 columns.

Comparison of Mono S 5/50 with Capto HiRes S 5/50

Figure 4 shows the comparison of separations of protein mixtures on Mono S 5/50 vs Capto HiRes S 5/50. The elution profiles are similar for these columns with Capto HiRes S having slightly higher resolution than the Mono S column.

Column:	Mono S 5/50 and Capto HiRes S 5/50
Sample:	1. Ribonuclease A 2. α-Chymotrypsinogen A 3. Cytochrome C 4. Lysozyme
Sample volume:	100 µL
Sample load:	0.5 mg
Binding buffer:	20 mM phosphate, pH 6.8
Elution buffer:	500 mM phosphate, pH 6.8
Gradient:	0% to 50% elution buffer in 20 CV
Flow rate:	1.0 mL/min

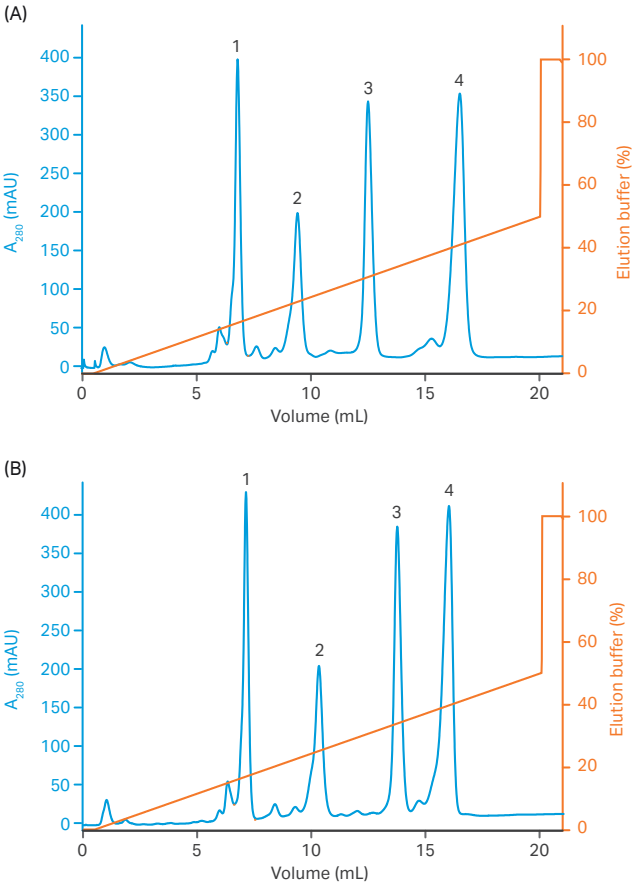


Fig 4. Separation of mixture of four proteins on (A) Mono S 5/50 and (B) Capto HiRes S 5/50.

Excellent reproducibility and durability

Reproducible results are essential in all research. The long working life and high reproducibility of Capto HiRes Q and Capto HiRes S are the result of optimized design, stable nature of the resin, and controlled production procedures.

Tests were performed to verify the chemical stability and robustness of the resin. A total of 300 purifications were carried out using cytochrome C and lysozyme by loading a normal preparative purification load of 5 mg to the column. A cleaning-in-place (CIP) procedure was performed every 10 cycles. Even after repeated protein purification cycles, cytochrome C and lysozyme peaks show very similar profiles (Fig 5), illustrating the very high robustness and reproducibility of the Capto HiRes S 5/50.

Column:	Capto HiRes S 5/50
Sample:	1. Cytochrome C, horse heart (1 mg/mL) 2. Lysozyme, chicken egg white (1 mg/mL)
Sample volume:	2.5 mL
Sample load:	5 mg
Binding buffer:	20 mM phosphate, pH 6.8
Elution buffer:	20 mM phosphate, 0.5 M NaCl, pH 6.8
Gradient:	40% to 50% elution buffer in 20 CV
Flow rate:	1.0 mL/min
CIP:	*Performed every 10 purification runs in order 2 M NaCl (2 CV); 1 M NaOH (4 CV); 2 M NaCl (2 CV). Flow rate, 1.0 mL/min*
System:	ÄKTA pure

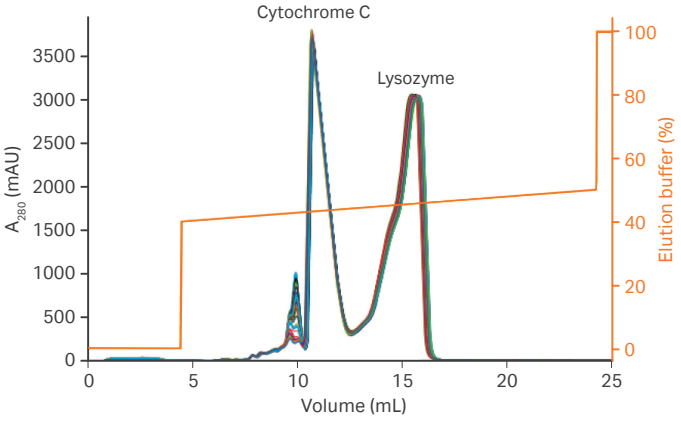


Fig 5. Overlay of chromatograms of 300 purification cycles using cytochrome C and lysozyme. Every 10th chromatogram is shown. A pre-peak before the main peak for cytochrome C is observed with increasing signal as the experiment progressed. This was caused by the small deterioration of the sample while stored in the refrigerator during the 2 wk that the experiments were ongoing.

Simultaneously, a functional test was performed using a standard protein mix. The four functional tests were performed at the start, after 100, 200 and 300 runs, showing very similar chromatograms (Fig 6), which demonstrates that the column performance was preserved after these 300 purification cycles.

Column:	Capto HiRes S 5/50
Sample:	1. Ribonuclease A, bovine pancrease (2.1 mg/mL) 2. α-Chymotrypsinogen A, bovine pancrease (0.7 mg/mL) 3. Cytochrome C, horse heart (1.1 mg/mL) 4. Lysozyme, chicken egg white (1.1 mg/mL)
Sample volume:	100 µL
Sample load:	0.5 mg
Binding buffer:	20 mM phosphate, pH 6.8
Elution buffer:	20 mM phosphate, 500 mM NaCl, pH 6.8
Gradient:	0% to 50% elution buffer in 20 CV
Flow rate:	1.0 mL/min
System:	ÄKTA pure

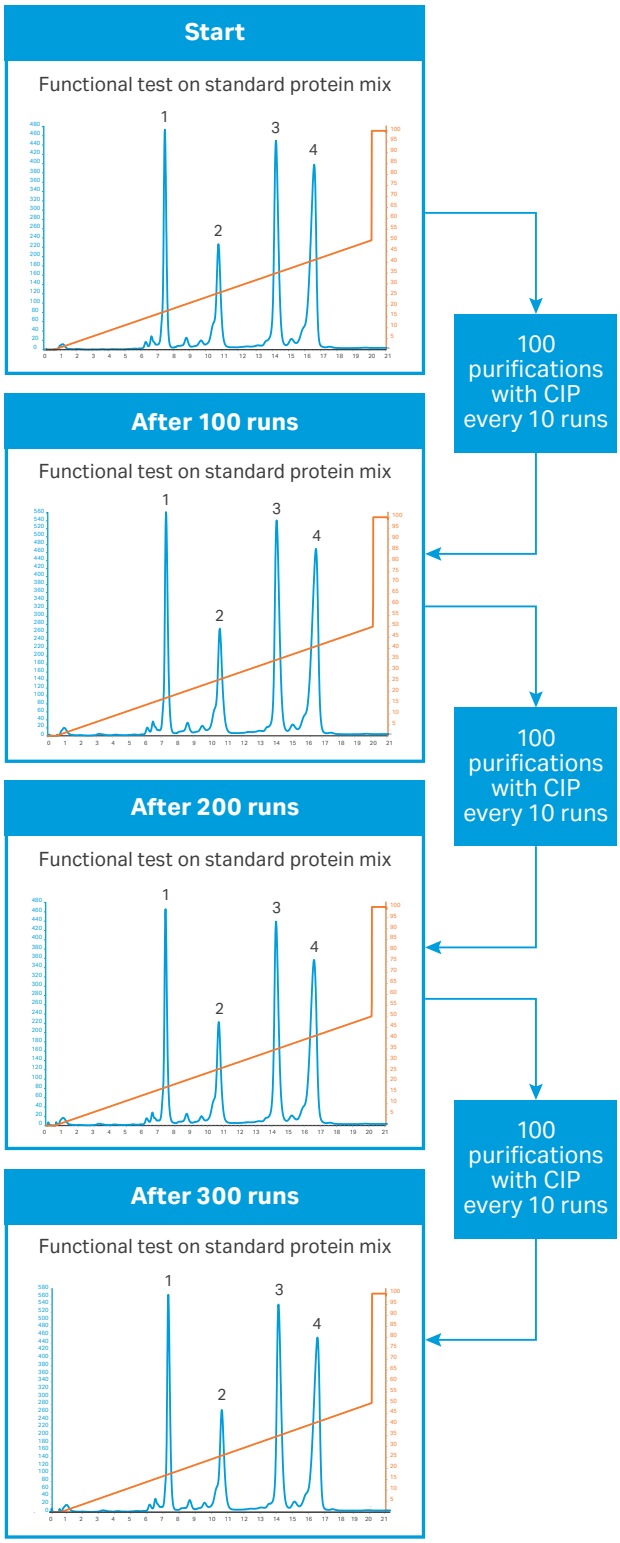


Fig 6. Functional test performed every 100 purifications.

Applications

Analysis of mAb charge variants on Capto HiRes S using pH gradient

For therapeutic monoclonal antibodies, various modifications can occur that can affect the biological activities of the antibodies. Thus, charge variants of these antibodies need to be monitored. Cation exchange chromatography is a standard method for analyzing charge variants of mAbs.

A monoclonal antibody was analyzed on an HPLC system using Capto HiRes S 5/50. The results are compared to the analysis results using Mono S 5/50. The mAb shows a similar charge profile on Capto HiRes S 5/50 (Fig 7).

Column:	Mono S 5/50 and Capto HiRes S 5/50
Sample:	A monoclonal antibody, 2.8 mg/mL in sample buffer
Sample load:	46 µg
Sample buffer:	10 mM NaH_2PO_4, 10 mM Tris, pH 6.4
Binding buffer:	10 mM sodium citrate, 10 mM sodium phosphate, 10 mM Tris, pH 5.0
Elution buffer:	10 mM sodium citrate, 10 mM sodium phosphate, 10 mM Tris, pH 9.0
Gradient:	40% to 100% in 10 CV
Flow rate:	0.5 mL/min
System:	HPLC

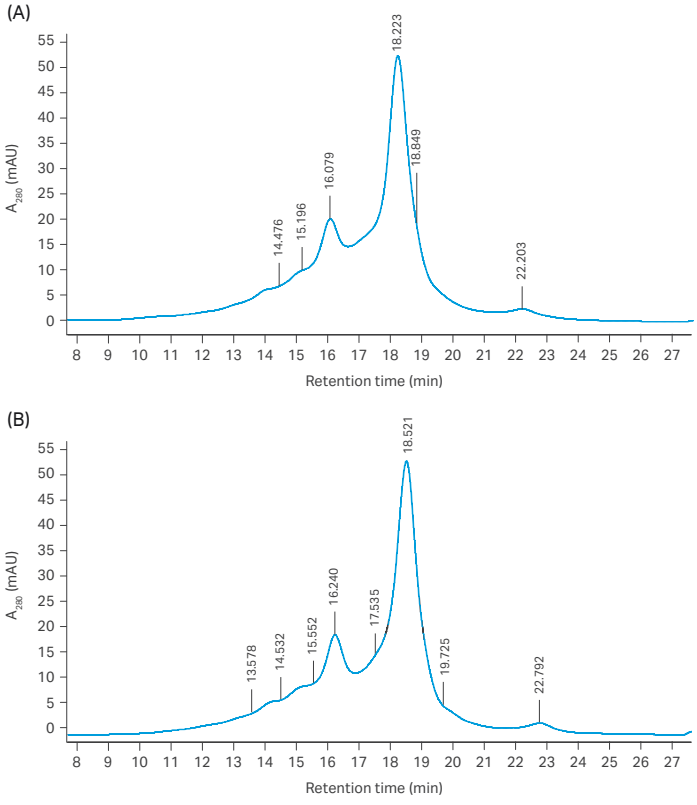


Fig 7. Charge profile of a mAb on (A) Mono S 5/50 and (B) Capto HiRes S 5/50.

Multistep purification using Capto HiRes Q between IMAC and SEC steps

FIP2 (Family of Interacting Protein 2) is a protein involved in recycling of endosomes to the plasma membrane. His-tagged FIP2 (residues 439–512) in active dimeric form with molecular weight (2 × M_r ~ 8000) was purified on an immobilized metal affinity chromatography (IMAC) with nickel (Ni^{2+}) ligand according to the procedure in Figure 8. The his-tag was then cleaved off the target protein after which the sample was run through a second Ni column to remove the uncleaved protein. Anion exchange chromatography (AIEX) using Capto HiRes Q 5/50 was performed in an intermediate purification step on an ÄKTApurifier chromatography system. The high-resolution column enables the separation of remaining uncleaved protein and charge variants from the pure protein (larger peak) (Fig 9). Fractions 17 and 18 from the main peak were pooled and further purified on a size exclusion chromatography (SEC) column. SDS-PAGE analysis of the fractions is shown in Figure 10. Uniform-shaped and well-diffracting crystals were then obtained from the final purified protein, as shown on Figure 11.

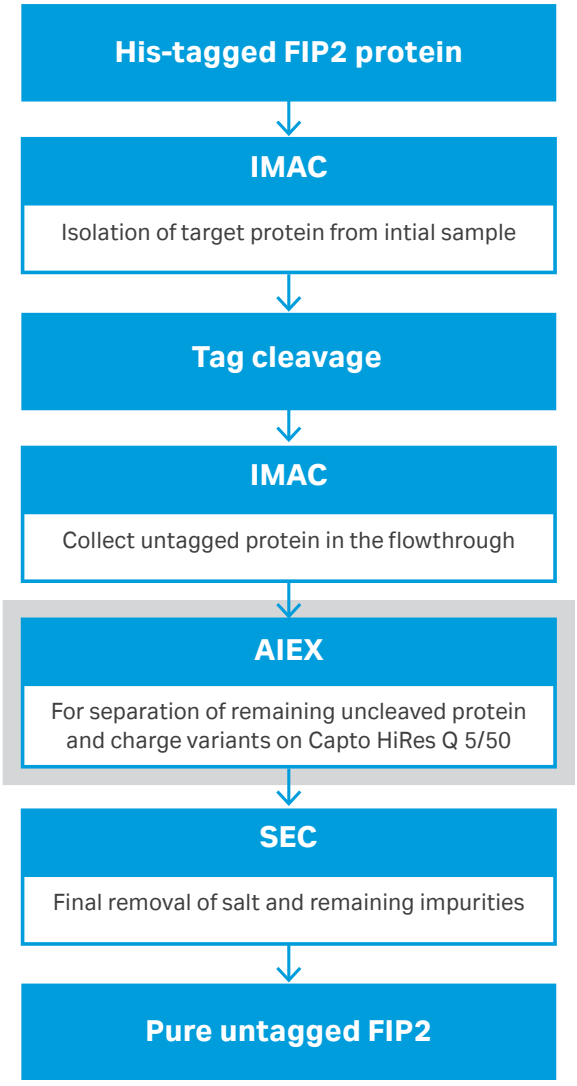


Fig 8. Summary of the experimental protocol.

Column: Capto HiRes Q 5/50
Sample: Partially purified his-tagged FIP2
Binding buffer: 10 mM Tris pH 8, 10 mM NaCl, 1 mM DTT
Elution buffer: 10 mM Tris pH 8, 1 M NaCl, 1 mM DTT
Gradient: 0% to 60% B in 30 CV
Flow rate: 1.0 mL/min
Fraction size: 0.5 mL
System: ÄKTApurifier

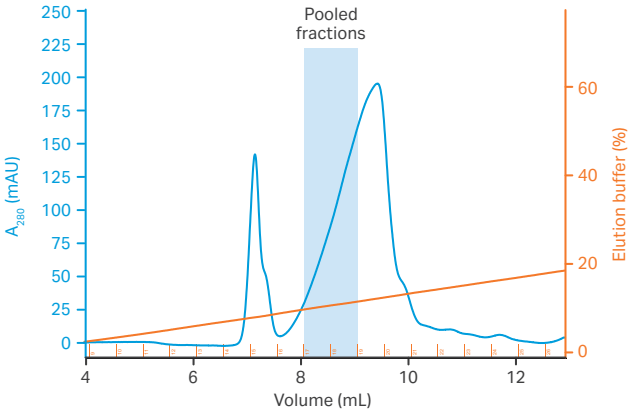
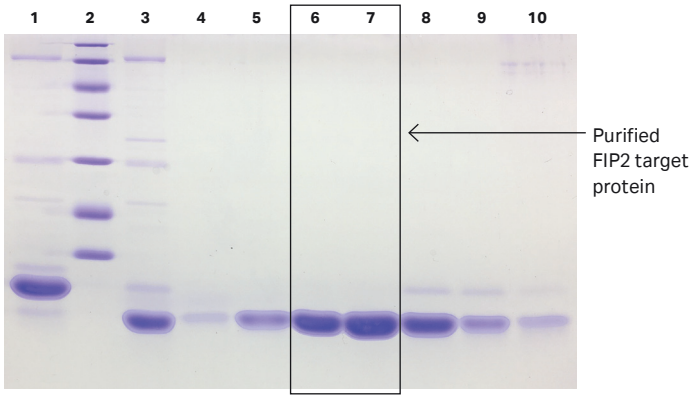


Fig 9. Capto HiRes Q 5/50 column separating the cleaved FIP2 protein from uncleaved proteins. The portion of the chromatogram containing FIP2 protein is shown in the shaded area.



Lane

1 Elute Ni-IMAC product; pre-enzymatic cleavage of his-tag
2 Molecular weight standards
3 Post-enzymatic cleavage of his-tag, sample loaded onto Capto HiRes Q 5/50
4–10 Fractions 15 to 20 from Capto HiRes Q 5/50 IEX run

Fig 10. SDS-PAGE analysis of FIP2 protein before and after ion exchange chromatography. Lanes 6 and 7, corresponding to fractions 17 and 18, contain purified FIP2 protein.

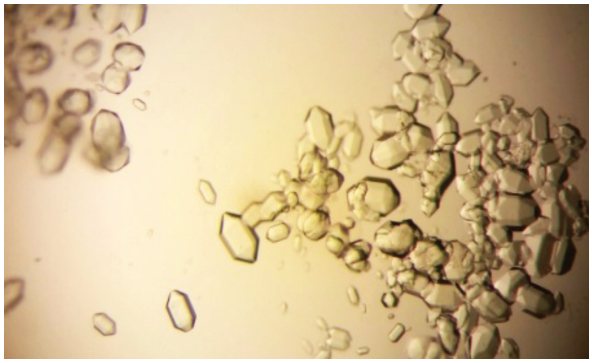


Fig 11. Crystals of FIP2 proteins purified on a multistep purification protocol using Capto HiRes Q 5/50 for the intermediate purification IEX step.

Preparative separation of single-stranded DNA

Purifications of oligonucleotides can often be carried out on an anion exchanger due to the negative charge of the phosphate group.

A large amount of single-stranded DNA (21mer) was synthesized on ÄKTA oligopilot plus oligonucleotide synthesizer. The oligonucleotides synthesized contained mainly 21mers but also DNA with other numbers of base pairs, thus further purification is usually needed for applications where high purity is required. Further purification of oligonucleotides can often be carried out on an anion exchanger. Here, 2 mg of a 21mer of single-stranded DNA was purified on a Capto HiRes Q 5/50 column, achieving a purity over 90%.

The initial purity of the oligonucleotides was analyzed after synthesis and the oligonucleotides from the synthesizer contained 76.5% of 21mers.

The pooled elution fractions of the IEX step (blue-shaded area, Fig 12) had a purity of 92.1% for 21mers making the ion exchange step useful in separating them from other oligonucleotides.

Column:	Capto HiRes Q 5/50
Sample:	Single-stranded DNA (21mer)
Sample load:	2 mg
Binding buffer:	50 mM Tris, pH 9.3
Elution buffer:	50 mM Tris, 1 M NaCl, pH 9.3
Gradient:	40% to 50% elution buffer in 20 CV
Flow rate:	0.5 mL/min
Fraction size:	0.5 mL
System:	ÄKTA pure 25

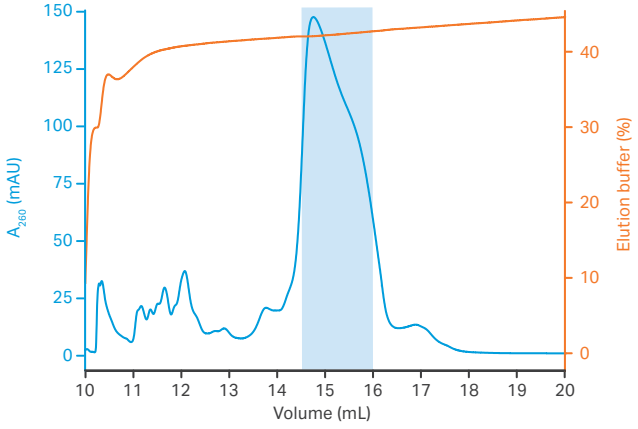


Fig 12. Separation of 21mer oligomers from other oligomers with varying base pair sizes. The 21mers were collected in the blue-shaded area of the chromatogram.

Acknowledgment

We thank Prof. Amir Khan at the Trinity College Dublin for the data on FIP2 protein purification.

Ordering information

Description	Pack size	Product code
Capto HiRes Q 5/50	5 × 50 mm	29275878
Capto HiRes S 5/50	5 × 50 mm	29275877
Capto HiRes Q 10/100	10 × 100 mm	29275881
Capto HiRes S 10/100	10 × 100 mm	29275879

Accessories

Product		
Tricorn 10 Filter Kit	1	29053612
Tricorn 5 Filter Kit	1	29053586
Filter tool	1	18115320
Fingertight connector, 1/16" male	10	18111255
Tricorn Storage/Shipping Device	1	18117643

Related literature	
Handbook: Ion exchange chromatography, principles and methods	11000421
Selection guide: Ion exchange chromatography columns and resins	18112731

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