Capto Chelating

AFFINITY CHROMATOGRAPHY

Capto™ Chelating chromatography medium (resin) is a BioProcess™ medium for immobilized metal ion affinity chromatography (IMAC) of native and histidine-tagged fusion proteins (Fig 1). The medium is suitable for both laboratory- and large-scale purifications. Capto Chelating is part of a platform of media based on the Capto product line.

Key benefits of Capto Chelating include:

- Suitable for a wide range of purification applications
- Straightforward scale-up to production columns
- Withstands effective and rigorous cleaning-in-place (CIP) procedures

Fig 1. Capto Chelating medium purifies proteins and peptides with affinity for chelated metal ions, including many proteins and peptides of commercial interest.

Introduction

Immobilized metal ion affinity chromatography

Certain amino acids, such as histidine, cysteine, and tryptophan, can form complexes with transition metal ions, for example, zinc, copper, and nickel. If any of these metal ions are immobilized onto a chromatographic medium by chelation with a suitable ligand, the medium will selectively retain proteins that expose these amino acids. This technique can thus be used to separate and purify proteins with affinity for chelating ions and is also the principle of IMAC, for which Capto Chelating has been designed.

IMAC is a very versatile purification technique. The possibility to select the metal ion not only provides the opportunity to work within the complete biologically relevant pH range, but also permits control of the selectivity of the medium. IMAC can be operated under a wide range of conditions, such as high salt concentrations as well as in the presence of chaotropic salts or denaturing agents. A wide range of biomolecules, including interferons, serum and plasma proteins, peptides, peptide hormones, lectins, and nucleotides, can be purified using IMAC (1, 2).

Characteristics

Capto Chelating consists of iminodiacetic acid groups coupled to a rigid agarose matrix by stable ether linkages and sufficiently long spacer arms (Fig 2). The highly cross-linked agarose base matrix gives the medium a high chemical and physical stability. The possibility to run at high flow velocities allows large volumes to be processed, which increases the productivity of large-scale bioprocessing operations. Capto Chelating is stable in conditions commonly used in process chromatography and cleaning procedures. Main characteristics of Capto Chelating are summarized in Table 1.

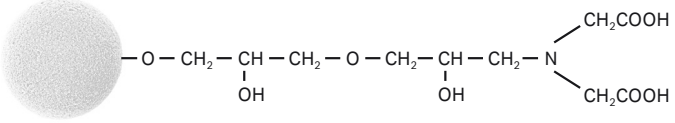

Fig 2. Partial structure of Capto Chelating. The rigid base matrix permits very high flow velocities.

Table 1. Main characteristics of Capto Chelating

Matrix	Highly cross-linked agarose
Copper capacity	23–33 µmol Cu^{2+}/mL medium
Particle size	75 µm (d_{50v})
Flow velocity	Minimum 600 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)
Chemical stability	Stable in commonly used aqueous buffers (three month study): 1.0 M NaOH 0.5 M NaOH 40% isopropyl alcohol 20% ethanol 6 M guanidine hydrochloride 8 M urea 0.01 M HCl
pH stability (operational)	3 to 12
CIP stability (short term)	2 to 14

Capto Chelating has excellent chemical stability under both acidic and alkaline conditions. The functional stability of the medium has been tested after storage at 40°C for seven days in 0.1 M HCl or 1.0 M NaOH and no significant change in performance was observed.

Experimental procedure

The following details highlight some key aspects of the experimental procedure.

Immobilization

Capto Chelating is supplied free of metal ions and thus needs to be charged with a suitable ion before use. When choosing the desired metal ion, consider the structural requirements underlying the basis of metal chelate-protein recognition. The metal ions predominantly used are Cu^{2+}, Zn^{2+}, and Ni^{2+}. Cu^{2+} ions bind strongly to a wide range of proteins and some proteins have a selective affinity for Cu^{2+}. Zn^{2+} ions generally give a weaker binding and in some cases, this can be exploited to achieve selective elution of a target protein. Ni^{2+} is frequently the metal ion of choice when working with proteins containing a histidine-tag. In some applications, Co^{2+}, Fe^{3+}, and Ca^{2+} have also been used advantageously. It is recommended that the medium is charged with the selected metal ion in distilled water to avoid precipitation of metal salts on the medium.

Binding and elution

Protein binding to an immobilized metal ion usually occurs in the pH range 5.5–8.5. Binding is often strongest at the upper end of this range. The choice of binding buffer depends on the chelated metal ion and on the binding properties of the sample molecules. Sodium acetate and sodium phosphate are recommended buffers.

Proteins may be desorbed from Capto Chelating by:

- Reducing pH, either continuous gradient or step-wise change. Most proteins elute between pH 6 and 4. A final pH of 3 to 4 is often suitable
- Competitive elution with gradient or step-wise increasing concentration of imidazole, histidine, ammonium chloride, or other substances with higher affinity for the chelated metal ion
- Chelating agents such as EDTA that will strip the metal ions from the medium and cause the proteins to co-elute. This method does not resolve different proteins

Eluting with imidazole or reducing pH suspends the interaction of the protein with the chelated metal ion, while the metal ion itself remains bound to the column. Histidine and ammonium chloride displace the metal:protein complexes from the iminodiacetic acid ligand by the chelating capacity of their primary amino functions.

Regeneration and cleaning

Before Capto Chelating can be recharged with a new metal ion, it should be regenerated by stripping the previously used metal ions from the medium in the packed column using EDTA.

In some applications, substances such as denatured proteins or lipids do not elute during regeneration. These can be removed in CIP procedures.

Cleaning in place

Recommended CIP procedures include:

- Removing ionically bound proteins by washing the column with 0.5 column volumes of a 2 M NaCl solution
- Removing precipitated proteins, hydrophobically bound proteins, and lipoproteins by washing the column with 1 M NaOH
- Removing strongly hydrophobically bound proteins, lipoproteins, and lipids by washing the column with 70% ethanol, 30% isopropanol, or detergents in a basic or acidic solution

Sanitization

Sanitization reduces microbial contamination of the medium. A recommended sanitization procedure is treatment with 0.5–1 M NaOH.

High flow rates and low back pressure in large scale

High flow velocities increase the productivity of large-scale bioprocessing operations and allow large volumes to be processed in one working shift. Capto Chelating is characterized by high mechanical stability and low back pressure to allow columns to be operated at high flow velocities with a wide range of bed heights at large scale (Fig 3). Typical flow velocities for Capto Chelating in a 1 m diameter column with 20 cm bed height are at least 600 cm/h, with a back pressure below 3 bar.

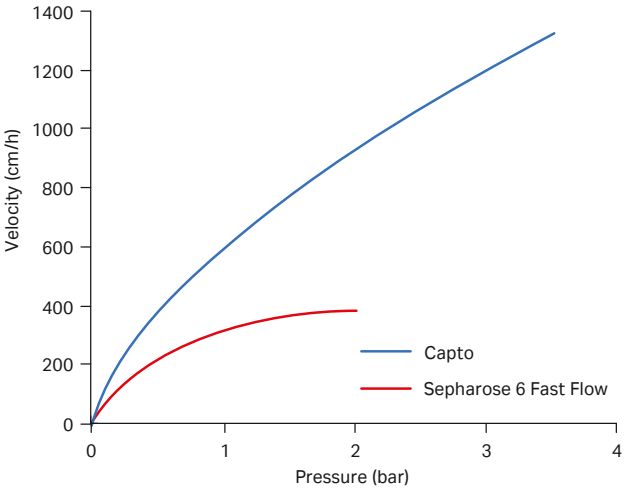

Fig 3. Pressure/flow properties of Capto Chelating compared with Sepharose 6 Fast Flow. Running conditions: BPG 300 (30 cm i.d.), open bed at settled bed height equal to 20 cm, with water at 20°C.

Productivity

A more rigid agarose medium allows for increased flow rates as well as the possibility to pack higher column beds, both enabling improved productivity. Increasing flow rate over the whole chromatographic purification process, (i.e., during column packing, conditioning, loading, washing, elution, regeneration, CIP, and reconditioning) can reduce total processing time substantially. With the possibility to pack higher column beds with retained diameter, more protein can be purified during the same cycle, enabling increased throughput. Altogether, using a more rigid chromatography medium such as the Capto medium can significant improve downstream process productivity.

Storage

Store used medium in the container at a temperature of 4°C to 30°C. Packed columns should be equilibrated in 20% ethanol to prevent microbial growth. After storage, equilibrate with at least five bed volumes of loading buffer before use.

Equipment

Capto Chelating is well suited for use with most equipment available for affinity chromatography, from laboratory to production scale. In process-scale, the preferred packing technique for Capto media is axial compression. The Intelligent Packing concept of AxiChrom™ columns offers preset packing methods for all Capto media. Suitable columns from Cytiva are listed in Table 2.

Table 2. Recommended columns for Capto Chelating at different scales of operations

Chromatography column	Inner diameter (mm)
Laboratory scale	
Tricorn™ columns	5, 10
HiScale™ columns	16, 26, 50
Pilot and production scale	
AxiChrom columns	50–1000
BPG columns	100–300
Chromaflow™ columns	400–800[1]

[1] Larger pack stations might be required for larger diameters.

Ordering information

Product	Pack size	Code number
Capto Chelating	25 mL	17-5485-01
Capto Chelating	100 mL	17-5485-02
Capto Chelating	1 L	17-5485-03
Capto Chelating	5 L	17-5485-04
Capto Chelating	10 L	17-5485-05
HiScreen™ Capto Chelating	4.7 mL	17-5485-10
HiTrap™ Capto Chelating	5 × 1 mL	17-5485-11
HiTrap Capto Chelating	5 × 5 mL	17-5485-12

Note: Capto Chelating bulk media are supplied in suspension in 20% ethanol.
For more information, contact your local Cytiva representative.

References

1. Kågedal, L., Immobilized Metal Ion Affinity Chromatography, in *Protein Purification, Principles, High Resolution Methods, and Applications*, 3rd edition (Jansson, J.-C. Ed). Wiley, Hoboken, New Jersey, pp. 183–201 (2011).
2. Chaga, G.S., Twenty-five years of immobilized metal ion affinity chromatography: past, present and future, *J. Biochem. Biophys. Methods* **49,** 313–334 (2001).

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For local office contact information, visit cytiva.com/contact

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