Capto Heparin

CUSTOM DESIGNED MEDIA

Capto™ Heparin, a medium for packed-bed affinity chromatography, gives fast and reliable separations of biomolecules with an affinity for heparin. These include coagulation factors, antithrombin III, and other plasma proteins, DNA binding proteins, lipoproteins, protein synthesis factors, and enzymes. Capto Heparin media is available as bulk media and in prepacked HiPrep™ columns.

This chromatography medium has several properties that make it particularly attractive for manufacturers seeking high productivity bioprocessing, including:

- Outstanding pressure and flow rate properties
- Excellent selectivity
- High chemical stability

Medium characteristics

Capto Heparin (Fig 1) is a chromatography medium based on porous spherical agarose particles (base matrix) with covalently attached heparin (ligand).

Heparin is a naturally-occurring glycosaminoglycan consisting of alternating hexuronic acid (D-glucuronic or L-iduronic) and D-glucosamine residues. The polymer is heavily sulfated, carrying sulfamino (N-sulfate) groups at C-2 of the glucosamine units as well as ester sulfate (O-sulfate) groups in various other positions (Fig 2). The heparin ligand in Capto Heparin is produced in compliance with cGMP for bulk pharmaceutical chemical manufacturers. It is isolated from porcine intestinal mucosa and the origin of the animals used is strictly controlled.

Capto Heparin is based on highly-rigid spherical agarose particles. Agarose is a natural polymer whose hydrophilic properties minimize structural changes of the target molecule and non-specific adsorption to the matrix. The rigid base matrix offers outstanding pressure and flow properties (Fig 3), which are key attributes for cost-effective, large-scale use. Capto Heparin permits a wide working range of flow velocities, bed heights, and sample viscosities, all of which have a positive impact on processing costs. Table 1 lists key characteristics of Capto Heparin.

Fig 1. Capto Heparin, which purifies proteins with an affinity for heparin, is available in laboratory and process-scale quantities.

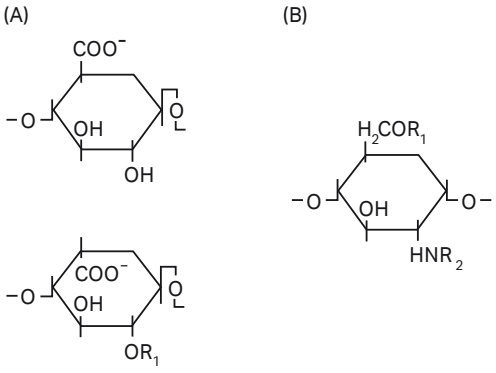

Fig 2. Heparin consists of alternating (A) hexuronic acid and (B) D-glucosamine residues. The hexuronic acid can be either D-glucoronic acid (top) or its C-5 epimer, L-iduronic acid (below). R1 = -H or $-SO_3^-$, R2 = $-SO_3^-$ or $-COCH_3$.

The heparin ligand is covalently bound to the agarose base matrix via a spacer. The coupling chemistry is reductive amination, and the resulting bond is stable even in alkaline conditions. Thus, the chemical stability of Capto Heparin is limited by the heparin ligand itself. Coupling is oriented to the reducing end of the heparin ligand. The ligand amount is optimized to give high selectivity for Factor IX.

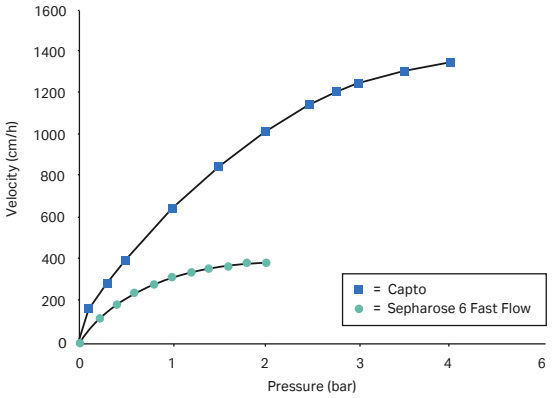

Fig 3. Open bed pressure/flow curve for the base matrix Sepharose™ 6 Fast Flow compared with the high-flow agarose base matrix used for Capto Heparin. Running conditions: BPG™ 300 column, open bed with water at 20°C at a settled bed height of 23 cm.

Table 1. Characteristics of Capto Heparin

Base matrix	Highly cross-linked spherical agarose
Average particle size[1]	90 µm (d_{50v})
Ligand	Heparin
Ligand density	Approx. 1.8 mg heparin/ml medium
Binding capacity	Approx. 1.4 mg ATIII/ml medium
Storage buffer	0.05 M sodium acetate containing 20% ethanol
pH stability	
Short-term[2]	4 to 13
Long-term[3]	4 to 12

[1] d_{50v} is the median particle size of the cumulative volume distribution.

[2] Short-term refers to the pH interval for regeneration and cleaning.

[3] Long-term refers to the pH interval where the medium is stable over a long period of time without adverse effects on subsequent chromatographic performance.

Chromatography equipment

Capto Heparin is well-suited for use with most equipment commonly used for affinity chromatography from laboratory to production scale. Due to the high rigidity of the medium, packing procedures differ slightly from Sepharose 6 Fast Flow media, for example. Recommended packing instructions are the same as for Capto Q. Table 2 shows suitable laboratory and process columns from Cytiva.

Table 2. Suitable chromatography columns

Column family	**Range (bed diameter)**
Tricorn™	5 mm, 10 mm
XK	16 mm
AxiChrom™	50 to 1000 mm

Principle of operation

Immobilized heparin has two chief modes of protein interaction: as an affinity ligand (e.g., its interaction with growth factors and antithrombin III), and as a cation exchanger due to its high content of anionic sulfate groups (e.g., its interaction with nucleic acid binding proteins where it mimics the polyanionic structure of nucleic acids). As individual proteins often bind by a unique combination of affinity and ion exchange, even small differences between bound proteins can result in good purifications.

Binding conditions for proteins that bind specifically to heparin are typically around physiological pH. To avoid non-specific interactions, the binding buffer needs an ionic strength of at least 0.15 M. However, if the target protein binds to heparin by cation exchange, a binding buffer of lower ionic strength might be more favorable.

Specific elution can be achieved by using heparin as competing agent. When heparin acts as a cation exchanger, elution is achieved simply by increasing the ionic strength of the eluting buffer. Continuous or step gradient elution using sodium chloride, potassium chloride, or ammonium sulfate at concentrations up to 1.5 to 2 M is commonly used.

Application

A typical protocol for using Capto Heparin to purify the blood coagulation factor antithrombin III from bovine plasma is shown below.

Sample:	80 ml bovine plasma mixed with 40 ml binding buffer
Binding buffer:	0.1 M Tris-HCl, 0.01 M trisodium citrate, 0.225 M NaCl, pH 7.4
Wash buffer:	0.1 M Tris-HCl, 0.01 M trisodium citrate, 0.330 M NaCl, pH 7.4
Elution buffer:	0.1 M Tris-HCl, 0.01 M trisodium citrate, 2.0 M NaCl, pH 7.4
Column:	1 ml Capto Heparin packed in a Tricorn 5/50 column

Chromatographic procedure

Flow rate:	0.5 ml/min
Equilibration:	5 column volumes (CV) binding buffer
Sample size:	45 ml prepared sample
Wash step 1:	40 CV binding buffer
Wash step 2:	15 CV wash buffer
Elution:	9.5 CV elution buffer

Cleaning-in-place

Substances such as denatured proteins that do not elute during regeneration can be removed by cleaning-in-place (CIP) procedures. A recommended CIP procedure for Capto Heparin is 4 CV of 0.1 M NaOH with a contact time of 1 to 2 h.

Sanitization

Sanitization reduces microbial contamination of the medium. A recommended sanitization procedure for Capto Heparin is to treat the packed column with 0.1 M NaOH and 20% ethanol for 1 h, or to allow it to stand in 70% ethanol for 12 h. Always wash the packed column with equilibration buffer after sanitization.

Storage

Store unused media at 4°C to 30°C in 20% ethanol and 0.05 M sodium acetate.

Ordering information

Product[1]	Quantity[2]	Code no.
Capto Heparin	25 ml	17-5462-01
Capto Heparin	200 ml	17-5462-02
Capto Heparin	1 l	17-5462-03
Capto Heparin	5 l	17-5462-04
HiPrep 16/10 Capto Heparin	1 × 20 ml	17-5462-14

[1] Part of the Custom Designed Media program from Cytiva.

[2] Larger quantities are available; please contact your local Cytiva representative.

Related literature	Code no.
Heparin Sepharose 6 Fast Flow, Data file	18-1060-76
Affinity Chromatography: Principles and Methods, Handbook	18-1022-29
Capto Q, Instructions	11-0026-19

cytiva.com/cdm

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