Affinity chromatography

Heparin Sepharose™ 6 Fast Flow

Heparin Sepharose 6 Fast Flow is a BioProcess™ resin for affinity chromatography (Fig 1). The resin allows fast and reliable separations of biomolecules with an affinity for heparin, including antithrombin III, coagulation factors and other plasma proteins, DNA binding proteins, lipoproteins, protein synthesis factors, enzymes that act on nucleic acids, and steroid receptors. The excellent flow characteristics and high chemical stability of Heparin Sepharose 6 Fast Flow make the resin highly suitable for process-scale purifications. As a member of the BioProcess family of resins. Heparin Sepharose 6 Fast Flow is well-supported with documentation that facilitates development, scale-up, and routine operation in production applications.

- Purification of heparin-binding biomolecules
- High chemical stability based on stable coupling chemistry
- Enhanced binding capacity for antithrombin III due to oriented coupling of the ligand
- Widely used in industrial applications and well-established in approved processes

Fig 1. Heparin Sepharose 6 Fast Flow purifies proteins with an affinity for heparin. As a BioProcess resin, the product is available in process-scale quantities.

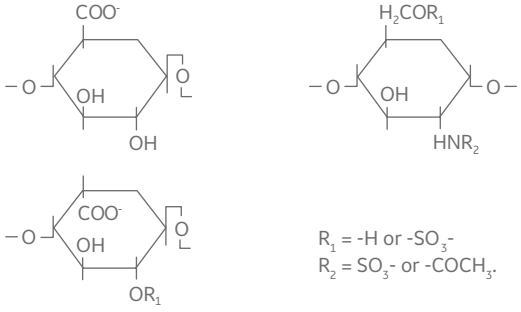


Fig 2. Heparin consists of alternating hexuronic acid (A) and D-glucosamine residues (B). The hexuronic acid can either be D-glucuronic acid (top) or its C-5 epimer, L-iduronic acid (below).

Characteristics

Heparin Sepharose 6 Fast Flow

Heparin is a naturally occuring glycosaminoglycan consisting of alternating hexuronic acid (D-glucuronic or L-iduronic) and D-glucosamine residues. The polymer is heavily sulphated, carrying sulphamino (N-sulphate) groups at C-2 of the glucosamine units as well as ester sulphate (O-sulphate) groups in various positions (Fig 2). The heparin is isolated from porcine intestinal mucosa, and has a molecular weight distribution of Mr 5000 to 30 000. The heparin ligand used for Heparin Sepharose 6 Fast Flow is produced in accordance with guidelines that comply with good manufacturing practise (GMP) for bulk pharmaceutical chemical producers. The heparin of animal origin is strictly controlled. Further information is available in the Regulatory Support File for Heparin Sepharose 6 Fast Flow.

The base matrix of Sepharose 6 Fast Flow is a robust, cross-linked 6% agarose.The crosslinking of the base matrix has been optimized to give the matrix good flow properties and high physical and chemical stability, both of which are key factors for cost-effective, large-scale use.

Linear flow velocities at process scale of 200 to 300 cm/h through a 15 cm bed height at a pressure of 1 bar (14.5 psi, 0.1 MPa) are easily achievable. Example of a pressure/flow curve is given in Figure 3. In many applications, lower flow velocities (e.g., 100 to 150 cm/h) are preferred to maximize binding conditions.

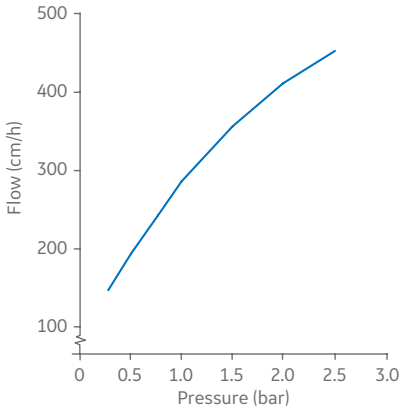


Fig 3. Pressure/flow curve for Heparin Sepharose 6 Fast Flow in BPG 200/500 column, inner diameter 20 cm, bed height 15 cm.

Heparin is linked to the Sepharose matrix by reductive amination and the resulting bond is stable even under alkaline conditions. Thus, the chemical stability of Heparin Sepharose 6 Fast Flow is limited only by the heparin ligand itself. Because of the oriented coupling of the heparin ligand and the used spacer, the specific binding activity is enhanced.

Table 1 lists the basic characteristics of Heparin Sepharose 6 Fast Flow.

Table 1. Characteristics of Heparin Sepharose 6 Fast Flow

Matrix	Cross-linked agarose, 6%, spherical
Particle size, d_{50v}[1]	~ 90 µm
Ligand	Porcine heparin
Ligand concentration	~ 2 mg heparin/mL resin
Pressure/flow characteristics	250-400 cm/h at < 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water)[2,3]
pH stability, operational[4] pH stability, CIP[5]	4 to 12 4 to 13
Chemical stability	Stable to commonly used aqueous buffers, 0.05 sodium acetate pH 4.0, 20% ethanol, 4 M NaCl, 8 M urea, 6 M guanidine hydrochloride, 0.1 M NaOH
Autoclavability	30 min at 121°C in in 0.02 M NaH_2PO_4 pH 7, 5 cycles
Delivery conditions	20 % ethanol containing 0.05 M sodium acetate
Storage	20 % ethanol containing 0.05 M sodium acetate, 4°C to 30°C

[1] Median particle size of the cumulative volume distribution.
[2] The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.
[3] Pressure/flow test performed on the base matrix.
[4] pH range where resin can be operated without significant change in function.
[5] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Experimental procedures

Recommendations and comprehensive instructions for packing and use are included in information supplied with Heparin Sepharose 6 Fast Flow. The following details highlight some key aspects of the operations.

Sanitization

Sanitization reduces microbial contamination of the resin. A recommended sanitization procedure is to treat the packed column with 0.1 M NaOH and 20% ethanol for 1 h. Always wash the packed column with equilibration buffer after sanitization.

Cleaning-in-place (CIP)

Substances such as denatured proteins that do not elute during regeneration can be removed by CIP procedures.

Heparin Sepharose 6 Fast Flow withstands exposure to 0.1 M NaOH for long periods without significant loss of binding capacity for antithrombin III. When contamination is severe, 0.5 M NaOH can be used. However, a decrease in functionality can be seen over time (Fig 4). Other reagents in which the resin is stable include 8 M urea and 6 M guanidine hydrochloride. Recommended CIP procedures are summarized in Table 2.

More detailed recommendations are included in the instructions enclosed with each pack of resin. Always wash the packed column with equilibration buffer after CIP.

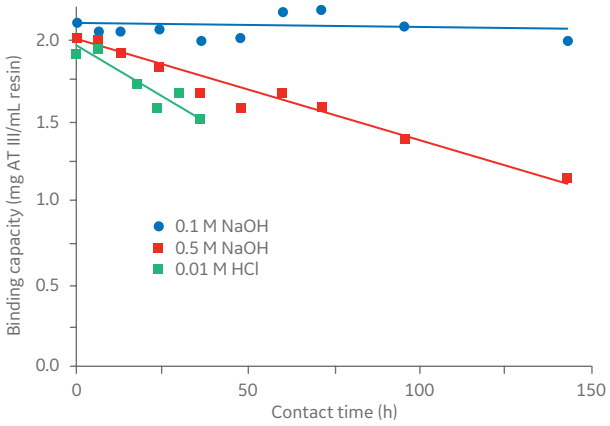


Fig. 4 Functional stability of Heparin Sepharose 6 Fast Flow was tested with three different CIP studies. CIP with 0.5 M NaOH can be used effectively over shorter periods.

Table 2. Recommended CIP procedures

For removal of	Wash with	Column volumes	Contact time
Ionically bound proteins	2 M NaCl	0.5	10–15 min
Precipitated or denatured proteins	0.1 M NaOH or 6M guanidine hydrochloride or 8 M urea	4 approx. 2 approx. 2	1–2 h 0.5–1 h 0.5–1 h
Hydrophobically bound proteins	non-ionic detergent	4	1–2 h

Equipment

Heparin Sepharose 6 Fast Flow is well-suited for use with most equipment commonly employed for affinity chromatography, from laboratory to production scale. Typical bed height for optimized performance, range from 10 to 20 cm.

Table 3 lists recommended empty columns from GE for laboratory use, scale-up, and production.

Table 3. Recommended columns for use with Heparin Sepharose 6 Fast Flow at different scales of operation

Column	Inner diameter (mm)	Approx. bed volume	Bed height (cm)
Lab scale			
Tricorn™ 5/20	5	up to 0.5 mL	max 2.6
Tricorn 5/50	5	0.2-1.1 mL	0.8–5.6
Tricorn 10/20	10	up to 2.1 mL	max 2.6
Tricorn 10/50	10	up to 4.4 mL	max 5.6
Tricorn 10/100	10	3.6–8.4 mL	4.6–10.6
HiScale™ 16/20	16	up to 40 mL	max 20cm
HiScale 16/40	16	16-80 mL	8.0–40
HiScale 26/20	26	up to 106 mL	max 20
HiScale 26/40	26	69-212 mL	13–40
HiScale 50/20	50	up to 393 mL	max 20
HiScale 50/40	50	274-785 mL	14–40
XK 16/20	16	up to 31 mL	max 15.5
XK 16/40	16	16-70 mL	8–35
XK 26/20	26	up to 66 mL	max 12.5
XK 26/40	26	45-186 mL	8.5–35
XK 50/20	50	up to 274 mL	max 14
XK 50/30	50	up to 559 mL	max 28
Production scale			
AxiChrom™	50–200	up to 16.7 L	max 50 cm
AxiChrom	300–1600	up to 1005 L	max 50 cm
BPG 100/500	100	0.3–2 L	1–26
BPG 140/500	140	0.1–4 L	1–26
BPG 200/500	200	0.3–8.1 L	1–26
BPG 300/500	300	0.6–17.2 L	3–25
BPG 450/500	450	25–35.9 L	16–23
INdEX 70/500	70	0.1–1.2 L	3–32
INdEX 100/500	100	0.2–2.5 L	3–32
INdEX 140/500	140	0.5–4.9 L	3–32
INdEX 200/500	200	0.9–10 L	3–32
Chromaflow™ 400/100–300	400	13–38 L	10–30
Chromaflow 600/100–300	600	30–86 L	10–30

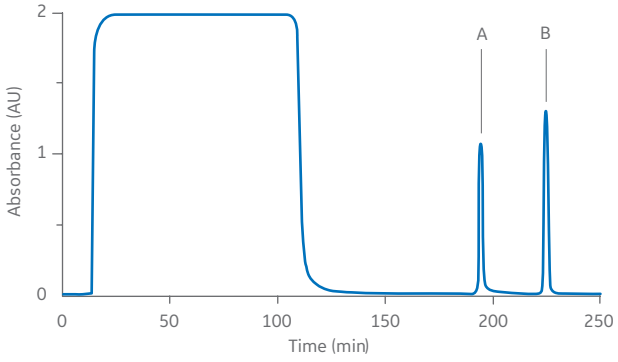
Application

Purification of antithrombin III

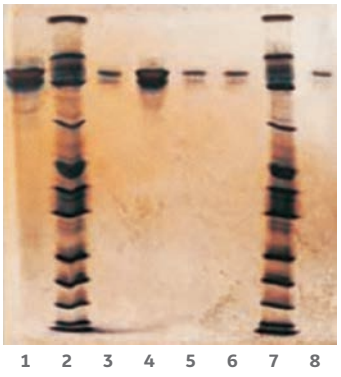
Purification of the blood coagulation protein antithrombin III from plasma and recombinant sources is a key industrial application for Heparin Sepharose 6 Fast Flow. One application example of the purification of antithrombin III from human plasma is given in Figure 5. In this example, the binding buffer was a citrate buffer and elution was accomplished by a step gradient of increasing ionic strength.

Sample: 45 mL pooled and filtered (0.45 µM) human plasma from 5 donors (0.45 µM) diluted 2:1 in 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: Heparin Sepharose 6 Fast Flow packed in HR 5/5 column

Chromatographic procedure:
Flow rate: 0.5 mL/min (150 cm/h)
Equilibration: 5 mL binding buffer
Washing step 1: 40 mL binding buffer
Washing step 2: 15 mL wash buffer
Elution: 9 mL elution buffer



Isoelectric focusing-PAGE analysis of the peaks A and B from the affinity chromatography.



Lanes 1 and 4: Peak B
Lanes 2 and 7: IEF calibration kit
Lanes 3 and 6: Antithrombin III (Sigma)
Lanes 5 and 8: Peak A

Fig 5. Purification of antithrombin III from human plasma on Heparin Sepharose 6 Fast Flow. Pure antithrombin III is present in both peak A and B.

Peak A elutes with washing buffer and peak B with elution buffer. Isoelectric focusing-PAGE analysis showed that pure antithrombin III was present in both these peaks. NORPartigen antithrombin III test of peaks A and B revealed a more active form of antithrombin III concentrated in peak B (data not shown). Thus, under the given conditions some antithrombin III with lower affinity for heparin was eluted in the washing step, while the more active antithrombin III was eluted in the elution peak. This difference in affinity can be used to separate the two forms of antithrombin III to obtain a highly active end product.

Technical support for process-scale use

For successful use of a chromatography resin in commercial production, rapid process development, smooth scale-up to production, and economic and trouble-free operation are essential. As a member of the BioProcess family of resins, Heparin Sepharose 6 Fast Flow fulfils these criteria. BioProcess resins meet the rigorous demands of successful downstream processing, including validated manufacture, secure supply, scalability, high productivity, effective sanitization/CIP, and regulatory support.

Evidence of this suitability can be found in numerous examples where purifications based on Heparin Sepharose 6 Fast Flow have been scaled up to reliable and economically viable production processes.

Ordering information

Designation	Quantity	Code number
Heparin Sepharose 6 Fast Flow	50 mL	17099801
Heparin Sepharose 6 Fast Flow	250 mL	17099825
Heparin Sepharose 6 Fast Flow	1 L	17099803
Heparin Sepharose 6 Fast Flow	5 L	17099804
Related literature		
Affinity Chromatography, Principles and Methods		18102229

gelifesciences.com/bioprocess

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752, USA
GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Japan Corp., Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan
GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

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