Heparin Sefinose™ 6 Fast Flow

Product information for BSP094-1/ BSP094-2：

Introductions

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. The heparin is isolated from porcine intestinal mucosa, and has a molecular weight distribution of Mr 5,000–30,000. Heparin Sefinose™ 6 Fast Flow is a BBI medium for affinity and ion exchange chromatography. It allows fast and reliable separations of biomolecules with an affinity for heparin, including coagulation factors, such as ATIII ,Factor IX,Factor VII, Factor XI, Factor XII, and XIIa, and other plasma proteins,DNA binding proteins, lipoprotein lipases, lipoproteins(LDL, VLDL, VLDL apoprotein, and HDL),enzymes that act on nucleic acids(DNA or RNA polymerases and restriction endonucleases and so on) and steroid receptors(Growth hormones).growth factors ,such as FGF and ECGF. The excellent flow characteristics and high chemical stability of Heparin Sefinose 6 Fast Flow make the medium highly suitable for process-scale purifications.

Characteristics

The base matrix, Sefinose 6 Fast Flow, is a robust, 6% highly cross-linked beaded agarose.The crosslinking of the base matrix has been optimized to give the matrix excellent flow properties, high physical and chemical stability, all of which are key factors for cost-effective, large-scale use, Linear flow rates at process scale of 200–300 cm/h through a 15 cm bed height at a pressure of 1 bar (14.5 psi, 0.1 MPa) are easily achievable. In many applications, lower flow rates, e.g. 100–150 cm/h, are preferred in order to maximize binding conditions. Heparin is linked to the Sefinose matrix by reductive amination and the resulting bond is stable even in alkaline conditions. Thus, the chemical stability of Heparin Sefinose 6 Fast Flow is limited only by the heparin ligand itself. Due to the oriented coupling of the heparin ligand and the used spacer, the specific binding activity is enhanced.

Bead structure	6% highly cross-linked spherical agarose
Mean particle size	90 µm (45–165 µm)
Ligand	heparin of porcine origin
Ligand density	approx. 4 mg/ml drained gel
pH stability	long term 4–12 , short term 4–13
hemical stability	0.1 M NaOH (1 week, +20 °C), 4 M NaCl, 8 M urea,0.05 M sodium acetate, pH 4.0,6 M guanidine hydrochloride
Pressure/flow	specification 200–400 cm/h, 1 bar, XK 50/60 column,bed height 25 cm (base matrix)
Autoclavable	121 °C for 20 minutes in distilled water
Storage buffer	0.05 M sodium acetate containing 20% ethanol

Prepacked column

Heparin Sefinose Fast 6 Flow is well-suited for use with most equipment commonly employed for affinity chromatography from laboratory to production scale. For best performance,we recommend bed heights of around 10–20 cm..some column ,such as Tricorn™ 10/100, XK 16/20 can be used for lab-scale for Heparin Sefinose 6 Fast Flow. BPG 140/500, chromaflow™ 400/100-300,BPG™ 100/500 column can be uesd for process-scale for Heparin Sefinose™ 6 Fast Flow. Heparin Sefinose 6 Fast Flow is supplied pre-swollen. Decant the 20% ethanol solution and replace it with binding buffer before use.

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Packing lab-scale columns

1. Assemble the column (and packing reservoir if necessary).
2. Remove air from the column dead spaces by flushing the end-piece and adaptor with packing buffer. Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with packing buffer.
3. Resuspend medium stored in its container by shaking (avoid stirring the sedimented medium). Mix the packing buffer with the medium to form 50–70% slurry (sedimented bed volume/slurry volume = 0.5–0.7).
4. Pour the slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles
5. If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adaptor or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adaptor or in the inlet tubing.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. Ideally, Sefinose 6 Fast Flow based media are packed at a constant pressure of approximately 1.5 bar (0.15 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 500 cm/h (10 cm bed height, 25°C , low viscosity buffer).If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate the pump can deliver. This should also give a reasonably well-packed bed.

   Note: Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures using the same pump.
7. When the bed has stabilized, close the bottom outlet and stop the pump.
8. If using a packing reservoir, disconnect the reservoir and fit the adaptor to the column.
9. With the adaptor inlet disconnected, push down the adaptor approximately 2 mm into the bed, allowing the packing solution to flush the adaptor inlet.
10. Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adaptor.
11. Close the bottom outlet. Disconnect the column inlet and lower the adaptor approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

Packing large scale columns

General packing recommendations

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully.

Pressure packing (BPG Columns)

BPG Columns are supplied with a movable adaptor. They are packed by conventional pressure packing by pumping the packing solution through the chromatographic bed at a constant flow rate (or back pressure).

1. Pour some water (or packing buffer) into the column. Make sure that there is no air trapped under the bottom bed support. Leave about 2 cm of liquid in the column.
2. Mix the packing buffer with the medium to form a 50–70% slurry.(sedimented bed volume/slurry volume = 0.5–0.7). Pour the slurry into the column. Insert the adaptor and lower it to the surface of the slurry,making sure no air is trapped under the adaptor. Secure the adaptor in place.
3. Seal the adaptor O-ring and lower the adaptor a little into the slurry, enough to fill the adaptor inlet with packing solution.
4. Connect a pump and a pressure meter and start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or medium.
5. When the bed has stabilized, close the bottom valve and stop the pump.The bed starts rising in the column. Loosen the O-ring and lower the adaptor to 0.5–1.0 cm above the bed surface.
6. Seal the O-ring, start the pump and continue packing.
7. Repeat steps 5 and 6 until there is a maximum of 1 cm between bed surface and adaptor when the bed has stabilized. Mark the bed height on the column tube.

8. Close the bottom valve, stop the pump, disconnect the column inlet and, without loosening the adaptor O-ring, push the adaptor down to approximately 3 mm below the mark on the column tube. The packing solution will flush the adaptor inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).

Note :As for evaluation of packing and columns packed in other ways,such as Hydraulic pressure packing(INdEX columns), Suction packing(BPSS Columns), chromaflow packing(chromaflow columns),please read and follow the relevant column instruction manuals carefully.

Binding

Immobilized heparin has two main modes of interaction with proteins.

Heparin Sefinose 6 Fast Flow can be used as an affinity chromatography media; e.g. for purification of coagulation factors or nucleic binding proteins. Different substances may differ in their affinity for Heparin Sefinose 6 Fast Flow. The binding capacity of a particular protein will depend upon parameters such as buffer composition, pH, flow rate and temperature.Heparin Sefinose 6 Fast Flow might also function as a cation exchanger due to the negatively charged sulphate and carboxylate groups on the immobilized heparin ligand.

A commonly used binding/equilibration buffer for the purification of plasma proteins is 10–20 mM sodium citrate buffer, Tris-HCl pH 7.4. Since the heparin ligand acts as an affinity ligand in these cases, it may be advisable to add 0.15M NaCl in order to eliminate unspecific ionic interactions. In other applications, if the protein of interest binds to heparin by ionic forces, a buffer of lower ionic strength may be used ,10 mM sodium phospate, pH 7.0 or 20 mM Tris-HCl, pH 8.0 are often recommended as binding/ equilibration buffers.

Elution

Elution is commonly performed by increasing the ionic strength of the buffer. Elution using a continuous linear gradient or step gradient with NaCl, KCl or $(NH_4)SO4$ up to 1.5–2 M is most frequently used. If some proteins may require severe conditions to elute from columns, specific eluants can also be used:chaotropic agents such as (0.5 M to 6 M) urea, guanidine, sodium thiocyanate; TritonX-100 (0.1-2%); ethylene glycol (0.1-2%); or pH shifts (use with care: from 3.2 to 10)

Regeneration

Depending of the nature of the sample, Heparin Sefinose may be regenerated for re-use by washing the medium with 2–3 bed volumes of alternating high pH (0.1 M Tris-HCl, 2.0 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate, 2.0 M NaCl, pH 5.0) buffers for removing ionically bound protein. This cycle should be repeated 3 times followed by Re-equilibrate the column with at least 3 bed volumes of binding buffer.

An alternative method for regeneration of the medium is to wash with a nonionic detergent for removing hydrophobic bound protein, e.g. 0.1% Triton X-100 at 37°C for one minute followed by re-equilibration with at least 3 bed volumes of binding buffer.

Cleaning-in-place

Substances such as denatured proteins that do not elute during regeneration can be removed by cleaning-in-place (CIP) procedures.Heparin Sefinose 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 will be seen over time . Other reagents in which the medium is stable include 8 M urea and 6 M guanidine hydrochloride.Recommended CIP procedures are summarized in Table, Always wash the column thoroughly with equilibration buffer after cleaning-in-place.

For removal of time	Wash with	Column volumes	Contact
Ionically bound proteins	2 M NaCl	0.5	10–15 min
Precipitated or	0.1 M NaOH	4	1–2 h
denatured proteins	or 6 M guanidine-HCl	approx. 2	30 min–1 h
	or 8 M urea	approx. 2	30 min–1 h
Hydrophobically	0.1–0.5% non-ionic	4	1–2 h
bound proteins	detergent		

A specific CIP protocol should be designed for each process according to the type of contaminants present. The frequency of CIP depends of the nature and the condition of the starting material, but one CIP cycle is generally recommended every 5 separation cycles.

Sanitization

For inactivation of microbial contaminants, equilibrate the column with buffer consisting of 0.1 M NaOH and 20% ethanol and allow to stand for 1 h.

Note: Alternatively, equilibrate with 70% ethanol and allow to stand for 12 h if working in an explosion-proof environment.Wash the column thoroughly with running buffer after sanitization.

Sterilization

Autoclaving is the only recommended sterilization treatment. Equilibrate the medium with 0.5 M NaCl, pH 7. Dismantle the column and autoclave the medium at 120 °C for 30 minutes. Sterilize the column parts according to the instructions in the column manual. Re-assemble the column, then pack and test it as recommended.

Storage

Store Heparin Sefinose 6 Fast Flow at +4 to 30°C in 0.05 M sodium acetate containing 20% ethanol as preservative.