Heparin Sefinose™ 6 FF Column

Product information for BSP093-1/ BSP0093-2/ BSP093-3/ BSP093-4；

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 EGF. The excellent flow characteristics and high chemical stability of Heparin Sefinose 6 Fast Flow make the medium highly suitable for process-scale purifications and also available in conveniently pre-packed 1ml, 5ml and 10ml gravity flow column .

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 term4–13
Chemical 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

Recommended Buffers

Equilibration buffer: 0.01 M Tris-HCl, pH 7.5 to 8.0 0.15 M NaCl

Note :other buffer systems may be substituted if the target protein is unstable in Tris buffers. Buffer additions are acceptable and at times essential for protein stability (i.e., mercaptoethanol, EDTA).

Note : Addition of salt to the Equilibration buffer reduces non-specific ionic interactions.It is

recommended to use 10 mM Tris-HCl pH 7.5 containing 0.15 M NaCl as Equilibration buffer. However, if the protein of interest binds to heparin by ionic forces, a buffer of lower ionic strength may be used.

Elution buffer: 0.01 M Tris-HCl, pH 7.5-8.0, with 1.5 M NaCl.

Note : alternative salts may be used (KCl, $CaCl_2$, NH_4Cl, $(NH4)_2SO4$). 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).

Sample preparation:

1. Centrifugation - eliminate particles by centrifuge and minimize lipid or lipoprotein content by delipid methods (this will aid in resin cleaning and extend column life).
2. Concentration - between 1-10 mg/ml.Equilibration to column conditions by dialysis, desalting columns, diafiltration, or dilution.

Procedure for Purification of Target Protein using a Gravity-flow Column

The total volume of the 1ml ,5ml and 10ml column is10ml, 30ml and 80ml ,respectively. If a sample volume is greater than the column, perform multiple applications until the entire sample has been processed. Be careful not to exceed the resin's binding capacity.

1. Equilibrate column to working temperature. Perform purifications at room temperature or at 4C.
2. Take out off the bottom cap, remove the top cap, pour off excess liquid and clamp the column and fix it on stand. Please let the top of the column is upturned.
3. Equilibrate column with 5-10 resin volumes of appropriate buffer for the target protein. Using a flow rate of 0.5-1 ml/minute, allow buffer to drain from resin.
4. Prepare sample by mixing the protein sample with 5 volumes of equilibration buffer. other ratios may be used, but need to be determined empirically.
5. Load the mixture of protein sample and equilibration buffer onto the column. Collect the flow-through in a tube. If desired, re-apply the flow-through once to maximize binding.
6. Wash the load into the column with 2 resin volume of equilibration buffer and collect the flow-through.
7. Continue washing to remove unbound protein.Washing may require 3-10 resin volumes for complete removal of free protein,until the absorbance of the flow-through fraction at 280 nm approaches baseline.
8. Elute bound protein with 2 resin volumes of chosen elution buffer. Repeat this step twice, collecting each fraction in a separate tube.
   Note: some proteins may require severe conditions to elute from columns.
9. Assay eluted fractions and the flow-through for target protein by measuring the absorbance of the fractions at 280nm or by BCA Protein Assay Kit(No SK3021) or Non-Interfering Protein Concentration Determination Kit (No SK3071). The eluted protein can be directly analyzed by SDS-PAGE.
   Note :Eluted fractions containing guanidine•HCl must be dialyzed against a buffer containing 8 M urea before SDS-PAGE analysis.
10. Clean and store the column.

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, 2.0M 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 Equilibration 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% TritonX-100 at 37°C followed by re-equilibration with at least 3 bed volumes of Equilibration buffer.

Cleaning-in-place

Substances such as precipitated or 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 that remove precipitated or denatured proteins, Always wash the column thoroughly with equilibration buffer after cleaning-in-place.

For removal of strongly bound impurities, the column can be washed with:

1. 2 resin volumes of 2 M ammonium sulfate
2. 3 resin volumes of 1 M Tris, pH 8.0, containing 6.0 M urea (or alternatively containing 6 M guanidine hydrochloride
3. 5 resin volumes of distilled water.

Then store column in 0.05 M sodium acetate containing 20% ethanol.

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.

Storage

This is a very stable agarose. If the resin is stored at 2-8°C in a buffer solution containing 0.02% thimerasol as a preservative, it should be useful and stable for up to 5 years. Do not freeze! Freezing will damage the agarose resin.