Heparin Beads 6FF

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1. Product Description

Heparin Beads 6FF is an affinity medium for purification of heparin-dependent biomolecules, 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 base matrix of **Heparin Beads 6FF** is highly cross-linked 6% agarose. The excellent flow characteristics and high chemical stability of **Heparin Beads 6FF** make the medium highly suitable for process-scale purifications.

Table 1. Characteristics of **Heparin Beads 6FF**

Item	Description
Matrix	Highly cross-linked 6% agarose
Ligand	Heparin sodium
Ligand density	>4 mg/ml medium
Particle size (μm)	45-165
Maxi pressure	0.3 MPa, 3 bar
pH stability	3-12
Storage buffer	1×PBS containing 20% ethanol
Storage	2-8℃

2. Purification Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be high purity. It is recommended filtering the buffers by passing them through a 0.22 μm or 0.45 μm filter before use.

Binding Buffer /Wash Buffer: 50 mM Tris-HCl, 10 mM sodium citrate, pH 7.4

Elution Buffer: 50 mM Tris-HCl, 10 mM sodium citrate, 1 M NaCl, pH 7.4

2.2 Sample Preparation

It is recommended filtering the sample solution by passing them through a 0.22 μm or 0.45 μm filter before use.

2.3Packing Columns

Heparin Beads 6FF is easy to pack and use, and its high flow properties make it excellent for industrial scaling-up. The method of packing the column is described below.

1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

2) Close the column outlet leaving the net covered with packing buffer.

3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **Boric Acid Beads 4FF** is packed at a constant pressure of approximately 3 bar (0.3 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer).If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5) When the bed has stabilized, close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6) With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

7) 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 adapter.

8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

2.4 Column Purification

1) Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the snap-off end at the column outlet.

2) Wash the column with 10 column volumes.

3) Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.

4) Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

5) Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

2.5 Analysis

Identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or western blot.

3. Cleaning-In-Place

In general, **PreCap Heparin** is well suited for reuse a number of times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium.

Remove the precipitation or denatured protein

Wash the column with 2 column volumes 0.1M NaOH or 6M guanidine hydrochloride solution. Finally wash the column with 5 column volumes 1×PBS (pH 7.4).

Remove the non-specific adsorption protein

Wash the column with 3 column volumes 70% ethanol or 1% Triton X-100.Finally wash the column with 5 column volumes 1×PBS (pH 7.4).

4. Related Products

Product	Cat. No.	Size
Heparin Beads 6FF	SA024005	5ml
	SA024025	25 ml
	SA024100	100ml
	SA024500	500ml
	SA02401L	1 L
	SA02410L	10 L
PreCap Heparin	SA024C11	1×1 ml
	SA024C51	5×1 ml
	SA024C15	1×5 ml
	SA024C55	5×5 ml
	SA024CS	3×1 ml+1×5 ml