GE Healthcare

Instructions 71-7078-00 AG **Affinity Media**

Heparin Sepharose CL-6B

Heparin Sepharose™ CL-6B is heparin from porcine intestinal mucosa immobilized by the CNBr method to Sepharose CL-6B.

Heparin is a highly sulphated glycosaminoglycan which serves as an anticoagulant by selectively binding antithrombin III. Heparin Sepharose CL-6B is a group specific adsorbent for affinity chromatography and is widely used for purifying serum proteins including coagulation factors, lipases, lipoproteins and hormone receptors. Heparin Sepharose CL-6B has also successfully been used for purifying of growth factors. The polyanionic structure of heparin, which serves as an analogue of DNA and RNA, has allowed purification of many types of proteins that interact with DNA or RNA, including polymerases, ligases, kinases, ribosomal proteins and some protein synthesis factors.

Sepharose™

Table 1. Medium characteristics

Ligand density:	~2 mg porcine Heparin/ml drained medium
Available capacity:	~2 mg antithrombin III/ml drained medium
Bead structure:	6% cross-linked agarose
Bead size range:	45–165 µm
Mean bead size:	90 µm
Max linear flow rate**:	150 cm/h at 25°C, HR 16/10 column, 5 cm bed height
pH stability***.	
Long term:	5–10
Short term:	5–10
Chemical stability:	Stable to all commonly used aqueous buffers and additives such as 8 M urea 6 M guanidine hydrochloride and detergents
Physical stability:	Negligible volume variation due to changes in pH or ionic strength.
Sanitization:	Sanitize the packed column with 2% Hibitane/20% ethanol or with 70% ethanol.

** Linear flow rate = $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$

*** The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration.

Contents

1. Preparing the medium

Heparin Sepharose CL-6B is supplied freeze-dried in the presence of additives. These additives must be washed away at neutral pH.

Weigh out the required amount of freeze dried powder (1 g freeze-dried powder gives about 4 ml final volume of medium) and suspend it in distilled water. The medium swells immediately and should be washed for 15 minutes with distilled water on a sintered glass filter. Use approximately 200 ml distilled water per gram freeze-dried powder, added in several aliquots.

Prepare a slurry with binding buffer in a ratio of 75% settled medium to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

2. Packing Sepharose CL-6B

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. De-gas the medium slurry.
3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate, see Table 1, is typically employed during packing.

Note: If you have packed at the maximum linear flow rate, do not exceed 75% of this in subsequent chromatographic procedures.

7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

3. Using an adaptor

Adaptors should be fitted as follows:

1. After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the medium bed is stable. Re-position the adaptor on the medium surface as necessary.

The column is now packed and equilibrated and ready for use.

4. Binding

Binding normally occurs around physiological pH.

Addition of salt to the binding buffer reduces non-specific ionic interactions. It is recommended to use 10 mM phosphate buffer pH 7.3 containing 0.15 M NaCl as binding buffer. However, if the protein of interest binds to heparin by ionic forces, a buffer of lower ionic strength may be used.

After sample application, the medium should be washed with 10 column volumes of binding buffer to remove unbound and non-specifically bound material.

5. Elution

As individual proteins often bind by a unique combination of specific affinity and/or ionic interactions, small differences between proteins can result in good purification factors.

Substances bound due to ionic interactions may be eluted by using an increasing salt gradient (linear or step). Normally, proteins elute at concentrations of salt lower than 1.5 M. Suitable elution salts are NaCl or KCl.

Specifically bound substances may be eluted by including heparin, 1–5 mg/ml, in the binding buffer.

6. Regeneration

Depending of the nature of the sample, Heparin Sepharose CL-6B may be regenerated for re-use by washing the medium with 2–3 bed volumes of alternating high pH (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate, 0.5 M NaCl, pH 5.0) buffers. This cycle should be repeated 3 times followed by re-equilibration in binding buffer.

Strongly adsorbed proteins can be removed by including 8 M urea or 6 M guanidine hydrochloride in the regeneration buffer.

Re-equilibrate the column with at least 5 bed volumes of binding buffer.

An alternative method for regeneration of the medium is to wash with a non-ionic detergent, e.g. 0.1% Triton™ X-100 at 37 °C for one minute followed by re-equilibration with at least 5 bed volumes of binding buffer. If necessary, 8 M urea or 6 M guandine hydrochloride may be added to the detergent solution.

Re-equilibrate with at least 5 bed volumes of binding buffer.

7. Sanitization

Sanitize the column by equilibrating it with a buffer consisting of 2% hibitane digluconate and 20% ethanol and allow it to stand for 6 hours. Alternatively, equilibrate the column with 70% ethanol and allow it to stand for 12 hours. In both cases, wash with at least 5 bed volumes of sterile filtered binding buffer at pH 7.0.

8. Storage

Freeze-dried Heparin Sepharose CL-6B should be stored below 8 °C.

Store swollen medium at 4–8 °C at neutral pH, in the presence of a bacteriostat, e.g. 20% ethanol. Swollen Heparin Sepharose CL-6B must not be frozen.

9. Further information

Check www.gehealthcare.com/protein-purification for more information. Useful information is also available in the Affinity Chromatography Handbook, see ordering information.

10. Ordering information

Product	Pack size	Code No.
Heparin Sepharose CL-6B	10 g	17-0467-01
Heparin Sepharose CL-6B	250 g	17-0467-09
Literature		
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29

www.gehealthcare.com/protein-purification
www.gehealthcare.com

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