GE Healthcare

Instructions 71-7094-00 AE Affinity Media

Gelatin Sepharose

Gelatin Sepharose™ 4B is gelatin coupled to Sepharose 4B by the cyanogen bromide method.

Gelatin binds specifically to fibronectin, which is a high molecular weight glycoprotein found on the surfaces of many cell types and present in many extracellular fluids including plasma. Gelatin Sepharose 4B has been designed for the purification or removal of fibronectin.

Sepharose™

Table 1. Medium characteristics.

Ligand density:	4.5–8 mg gelatin/ml drained medium
Available capacity:	approx. 1 mg human plasma fibronectin/ml drained medium
Bead structure:	4% agarose
Bead size range:	45–165 µm
Average bead size:	90 µm
Max linear flow rate*:	75 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
pH stability**:	
Long term:	3–10
Short term:	3–10
Chemical stability:	Stable to all commonly used aqueous buffers
Physical stability:	Negligible volume variation due to changes in pH or ionic strength
Storage:	20% ethanol

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

** 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 gel 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 and cleaning.

1. Preparing the medium

Gelatin Sepharose 4B is supplied pre-swollen in 20% ethanol. Prepare a slurry by decanting the ethanol solution and replacing it 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 4B medium

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

Fibronectin binds to Gelatin Sepharose 4B at or around physiological pH and ionic strength. Phosphate or Tris-HCl buffered saline have been commonly used as binding buffer for purification or removal of fibronectin.

Since fibronectin has a tendency to adsorb to glass, it is

recommended to only use siliconized glass to prevent adsorption and loss or fibronectin.

After the sample has been loaded, wash the medium with binding buffer until the base line is stable.

5. Elution

Fibronectin can be eluted from Gelatin Sepharose 4B in different ways.

- Use a buffer containing a bromide salt, e.g. sodium bromide or potassium bromide, and pH lower than the binding buffer. A recommended buffer is 0.05 M sodium acetate, pH 5.0, containing 1.0 M sodium bromide or potassium bromide.
- Elute adsorbed fibronectin with 8 M urea in the binding buffer.
- Fibronectin can also be eluted by adding arginine to the binding buffer.

6. Regeneration

Depending of the nature of the sample, Gelatin Sepharose 4B 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 4.5) buffers. This cycle should be repeated 3 times followed by re-equilibration with 3-5 bed volumes of binding buffer.

If detergent or denaturing agents (*e.g.* 8 M urea) have been used during the purification, these can also be used in the washing buffer.

7. Cleaning

In some applications, substances like denaturated proteins or lipids do not elute in the regeneration procedure. These can be removed by washing the medium with a detergent solution, *e.g.* 0.1% Triton™ X-100 at 37 °C for one minute.

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

8. Storage

Gelatin Sepharose 4B should be stored in neutral pH at 4–8 °C in presence of a bacteriostat, *e.g.* 20% ethanol.

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.
Gelatin Sepharose 4 B	25 ml	17-0956-01
Gelatin Sepharose 4 B	500 ml	17-0956-03

Literature		
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29

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

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