Gelatin Sepharose™ 4B

In Gelatin Sepharose 4B, gelatin is coupled to the Sepharose 4B by a cyanogen bromide coupling method.

Gelatin has a strong affinity to fibronectin, which is a high molecular weight glycoprotein found on the surfaces of many cell types and also present in many extracellular fluid including the plasma.

Gelatin Sepharose 4B is designed for the purification or removal of the fibronectin.

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Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 BioProcess™ resins

Gelatin Sepharose 4B belongs to the BioProcess resins. BioProcess chromatography resins are developed and supported for production-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Resin characteristics

Ligand concentration	~ 4.5 to 8 mg gelatin/mL resin
Matrix	Agarose, 4% spherical
Particle size, d_{50V} [1]	~ 90µm
Recommended maximum operating flow velocity [2]	75 cm/h
pH stability, operational [3]	4 to 9
pH stability, CIP [4]	4 to 9
Chemical stability	Stable to commonly used aqueous buffers, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol
Physical stability	Negligible volume variation after changes in pH or the ionic strength
Storage	20% ethanol, 2°C to 8°C

1 Median particle size of the cumulative volume distribution.

2 In a HR 16/10 column with 16 mm diameter and 5 cm bed height using buffers with the same viscosity as water at 25°C.

3 pH range where resin can be operated without significant change in the function.

4 pH range where resin can be subjected to cleaning or sanitization in place without significant change in function.

3 Preparing the resin

Gelatin Sepharose 4B is supplied preswollen in a 20% ethanol solution.

Step	Action
1	Decant the ethanol solution.
2	Replace the ethanol with a binding buffer.

Note:

The column can be equilibrated with a viscous buffers at low flow rates after the packing is completed.

Note:

The slurry must be in a ratio of 75% settled resin to 25% binding buffer. The binding buffer must not contain agents that significantly increase the viscosity.

4 Packing procedure

Step	Action
1	Heat all the material to the temperature at which the chromatography is performed.
2	De-gas the resin slurry.
3	Remove the air from the column by flushing the end piece of the column with the buffer. ***Note:*** *Make sure no air has been trapped under the column net.*
4	Close the column outlet with a few centimeters of buffer remaining in the column
5	Pour the slurry down a glass rod into the column in one continuous motion. ***Note:*** *Pouring the slurry down a glass rod and against the wall of the column minimizes the creation of air bubbles.*
6	Immediately fill the rest of the column with the buffer.

Step	Action
7	Connect the column top piece onto the column and connect the column to a pump.
8	Open the bottom outlet of the column and set the pump to run at the required flow rate. ***Note:*** *The flow rate must be at least 133% of the flow rate to be used during the subsequent chromatographic procedures. The maximum flow rate is typically employed during the packing, see* *Resin characteristics, on page 3**.* ***Note:*** *If you have packed the column at the maximum flow velocity, do not exceed 75% of that flow velocity in the subsequent chromatographic procedures.*
9	Maintain the packing flow rate for 3 bed volumes when a constant bed height is reached.

Using a packing adapter

Fit a packing adapter as follows:

Step	Action
1	After the resin has been packed, close the column outlet and remove the top piece from the column.
2	Carefully fill the rest of the column with buffer to form an upward meniscus at the top of the column.
3	Insert the adapter into the columnat an angle of 45° so that no air is trapped under the net.
4	Slide the plunger slowly down the column so that the air above the net and in the capillary tubing is displaced by the eluent. ***Note:*** *Open the valves in the inlet of the column to remove any air.*
5	Lock the adapter in position on the resin surface.
6	Open the column outlet and start the eluent flow.
7	Allow the eluent to flow through the column at the packing flow rate until the resin bed is stable.
8	Reposition the adapter on the resin surface as necessary. *Result*: The column is now packed, equilibrated, and ready to use.

5 Binding

Fibronectin binds to Gelatin Sepharose 4B at or around physiological pH and ionic strength. Phosphate or Tris-HCl buffered saline is commonly used as binding buffer for purification or removal of fibronectin. Since fibronectin has a tendency to adsorb to glass, it is recommended to use only siliconized glass to prevent the adsorption and loss of fibronectin.

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

6 Elution

Fibronectin can be eluted from Gelatin Sepharose 4B using:

- A buffer containing a bromide salt, for example, sodium bromide or potassium bromide with pH lower than the binding buffer
  Note:
  A recommended buffer is 0.05 M sodium acetate, pH 5.0, containing 1.0 M sodium bromide or potassium bromide.
- 8 M urea in the binding buffer
- Arginine in the binding buffer

7 Regeneration

Depending on the nature of the sample, Gelatin Sepharose 4B can be regenerated for reuse as follows:

Step	Action
1	Wash the resin with 2 to 3 bed volumes of 0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5
2	Wash the resin with 2 to 3 bed volumes of 0.1 M sodium acetate, 0.5 M NaCl, pH 4.5
3	Repeat step 1 and 2, three times
4	Re-equilibrate the washed resin with 3 to 5 bed volumes of the binding buffer.

Detergents or denaturing agents (e.g., 8 M urea) that have been used during the purification, can also be reused in the washing buffer.

8 Cleaning

In some applications, substances like denaturated proteins or lipids do not elute by the regeneration. These substances can be removed by washing the resin using a detergent solution, at 37°C for one minute. Re-equilibrate the resin then immediately with at least 5 bed volumes using the binding buffer.

9 Storage condition

Store unused or packed Gelatin Sepharose 4B as follows:

When	Then
Unused resin	Store the unsued resin in its container at 2°C to 8°C, and check that the screw top is fully tightened.
Packed column	Equilibrate the packed column in a buffer containing 20% ethanol to prevent microbial growth.

10 Further information

Further information is available in the Affinity Chromatography Handbook, see *Section 11 Ordering information, on page 9*.

11 Ordering information

Product	Quantity	Product code
Gelatin Sepharose 4 B	25 mL	17095601
Gelatin Sepharose 4 B	500 mL	17095603
Gelatin Sepharose 4 B	5 L	17095604

Literature	Quantity	Product code
Affinity Chromatography Handbook Vol. 3: Specific Groups of Biomolecules	1	18102229

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