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Gelatin-Sepharose 4B

Product Number: BSGS-25ml

Shipping and Storage

Store in a sealed environment at 4-8℃. After use, rinse thoroughly with pure water and store in 20% ethanol at 4-8℃.

Description

Gelatin specifically binds to fibronectin, a high molecular weight glycoprotein found on the surface of many cell types and present in many extracellular fluids, including plasma. Gelatin agarose gel 4B has been designed to purify or remove fibronectin.

Technical indicators

Product name	Gelatin-Sepharose 4B
Substrate	4% agarose gel
Particle size	45-165μm
Ligand density	About 4-6 mg gelatin/ml filler
Maximum flow rate	50 cm/h
Pressure resistance	0.1 Mpa
pH stability	3-10
Chemical stability	All commonly used aqueous buffer solutions

Testing conditions: chromatography column 10mm × 200mm; column bed height 5cm, 25℃

Protocol

1. Sample preparation

Before loading the sample, it should be filtered or centrifuged using a 0.45μm filter. If the sample is too viscous, dilute it with binding buffer to prevent clogging of the chromatographic column.

2. packing

2.1. Let all materials and reagents equilibrate to the temperature of the chromatography experiment. Prepare buffer solution and degas all buffer solutions.

2.2. Check all components of the chromatography column, especially the filter screen, sealing ring, screw plug for tightness, and glass tube for cleanliness and integrity.

2.3. Measure the corresponding amount of gel as required, and wash off 20% ethanol with deionized water.

2.4. Wet the bottom of the column with water or buffer solution and maintain a small liquid level, making sure there are no bubbles at the bottom.

2.5. Use a glass rod to guide the homogenate along the inner wall of the column and pour it into the column at once, taking care not to generate bubbles. Open the liquid outlet of the column to make gel

2.6. Allow free settlement within the column and connect the top column head of the column.

3. Equilibrium chromatography column

Equilibrate the column with 5-10 column volumes of binding buffer until the conductivity and pH of the effluent remain unchanged. All buffer solutions need to be filtered using a 0.45μm filter.

4. Sample loading

The sample should be prepared with equilibrium solution, and the sample must be centrifuged or filtered before loading. Samples with high salt concentration need to be processed before mixing.

Fibronectin binds to gelatin agarose gel 4FF near its stable pH and ionic strength. Phosphate buffer or Tris HCl buffer is usually used as the binding buffer for purification or removal of fibronectin.

5. Elution

Use elution buffer and stepwise gradient or linear gradient elution. For the elution step, 5 column volumes of elution buffer are usually sufficient. For linear gradient elution, increase the elution steps appropriately.

Fibronectin can be eluted from gelatin agarose gel 4FF in different ways:

5.1. Use a buffer containing bromide salts, such as sodium bromide or potassium bromide, with a pH lower than the binding buffer. The recommended buffer is 0.05M sodium acetate, pH 5.0, Contains 1.0M sodium bromide or potassium bromide.

5.2. Add 8M urea to the binding buffer to elute the adsorbed fibronectin.

5.3. Fibronectin can also be eluted by adding arginine to the binding buffer.

Regeneration

The gelatin agarose 4FF can be regenerated by alternately washing the packing with high pH (0.1M Tris HCl, 0.5M NaCl, pH 8.5) buffer and low pH (0.1M sodium acetate, 0.5M NaCl, pH 4.5) buffer of 2-3 column bed volumes. This cycle should be repeated 3 times and then equilibrated with 3-5 times the column bed volume of binding buffer.

Note

1. Before loading, the sample must undergo membrane filtration and removal of pigments, otherwise impurities and pigments will be adsorbed onto the filler, affecting its normal use. All buffer solutions need to be filtered through a 0.45μm filter.
2. During use, avoid using high concentrations of strong acids and bases. The concentration of acids and bases should be less than 0.1 moles. Alkali will slow down the flow rate.
3. Different samples have different adsorption and elution methods, which can be determined based on relevant literature.