Gelatin Beads 4FF

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1. Product Description

Gelatin Beads 4FF is a kind of meduim used in purification and removing fibronectin. Fibronectin is a large glycoprotein which widely exist in animal tissues, tissue fluids and plasma. **Gelatin Beads 4FF** using high crosslinking 4% agarose, which can tolerate high flow velocity and have chemical stability. **Gelatin Beads 4FF** are suitable for large-scale purification.

Table 1. Characteristics of **Gelatin Beads 4FF**

Item	Description
Matrix	Highly cross-linked 4% agarose
Ligand	gelatin
Ligand density	>4mg/ml medium
Binding capacity	1mg Human plasma fibronectin
Particle size (μm)	45-165
Maxi pressure	0.3 MPa, 3 bar
pH stability	3-10
Storage buffer	1XPBS containing 20% ethanol
Storage	2°C - 8°C

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 0.22μm or 0.45 μm filter before use.

Binding /Wash Buffer: 50mM Tris-HCl, 0.15M NaCl, 1mM EDTA, pH7.4

Elution Buffer: 50mM sodium acetate , 1.0M NaBr or KBr, pH5.0

The optional Elution Buffer: Binding Buffer with 8M Urea

The pH and ion concentration of Binding /Wash Buffer can be selected under physiological conditions, either phosphate or Tris-HCl buffer can be chosen.

2.2 Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascites or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight against Binding/Wash Buffer.

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

Note: fibronectin can adsorb to the glass. Silicified glass can be used in containers to reduce sample loss.

2.3 Packing of Column

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,**Gelatin Beads 4FF** is packed at a

constant pressure of approximately 0.3bar (0.3MPa). 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 Sample 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 of binding buffer .

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

Wash the column with 3 column volumes 0.1M Tris-HCl， 0.5M NaCl, pH8.5 and 0.1 M NaAc, 0.5M NaCl, pH4.5, repeat the step for 3 times, followed by washing the column with 5 column volumes of Binding Buffer.

Remove the precipitation or denatured protein

Wash the column with 0.1% Triton X-100 at 37°C. And then washing the column with 5 column volumes of Binding Buffer.

4. Related Products

Product	Cat. No.	Size
Gelatin Beads 4FF	SA054005	5 ml
	SA054025	25 ml
	SA054100	100 ml
	SA054500	500 ml
	SA05401L	1 L
	SA05410L	10 L