Blue Beads 6FF

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1. Product Description

Blue Beads 6FF is a chromatography medium for the isolation of proteins， such as albumin, interferon, and blood coagulation factors. **Blue Beads 6FF** also binds several enzymes including kinases, dehydrogenases, and most enzymes requiring denyl-contatining cofactors, for example NAD+.

Blue Beads 6FF consists of 90μm beads of highly cross-linked 6% agarose, is highly stable and expand the range of suitable operating conditions.

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

Item	Description
Matrix	Highly cross-linked 6% agarose
Ligand	Cibacron Blue 3G
Capacity (/ml medium)	>18 mg bovine serum albumin
Particle size (μm)	45-165
Maxi pressure	0.3 MPa, 3 bar
pH stability	4-12
Storage buffer	0.1 M K_3PO_4, 20% ethanol, pH 8.0
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 0.45μm filter before use.

Purification of human serum albumin as an example.

Binding /Wash Buffer: 50mM Na_2HPO_4, 50mM Na-Citrate, pH7.0

Elution Buffer: 50mM Na_2HPO_4, 50mM Na-Citrate, 1-2M NaCl, pH7.0

The Binding/Wash Buffer and Elution Buffer can be changed according to the properties of the samples. The principle is load at low salt and elute at high salt.

2.2 Sample Preparation

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

2.3 Packing Columns

Blue Beads 6FF are widely used in industrial purification. Therefore, it involves the packing of various medium and low pressure chromatographic columns. The following describes the packing method of **Blue Beads 6FF** 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, **Blue Beads 6FF** is packed at a constant pressure of approximately 3 bar (0.3 MPa). 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 at least 5 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 10 to 15 column volumes of binding buffer or until no material appears in the effluent.

5). Elute with 5-10 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

In general, **Blue Beads 6FF** is well suited for reuse a number of times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium as follows.

Remove the precipitated proteins or denatured substance

Wash the column with 3-4 column volumes 0.1M NaOH, followed by washing the column with 3-4 column volumes of 70% ethanol or 2M potassium thiocyanate. Alternatively, wash the column with 2 column volumes of 6M guanidine hydrochloride.

Finally wash the column with at least 5 column volumes of binding buffer.

Remove the strong hydrophobic binding proteins, lipoproteins and lipids

Wash the column with 3-4 column volumes 70% ethanol. Alternatively, wash the column with two column volumes of detergent in a basic or acidic solution. Wash the residual detergent by 5 column volumes of 70% ethanol.

Finally wash the column with at least 5 column volumes of binding buffer.

4. Related Products

Product	Cat. No.	Size
Blue Beads 6FF	SA027005	5 ml
	SA027025	25 ml
	SA027100	100 ml
	SA027500	500 ml
	SA02701L	1 L
	SA02710L	10 L
PreCap Blue	SA027C11	1X1ml
	SA027C51	5x1ml
	SA027C15	1X5ml
	SA027C55	5X5ml
	SA027CS	3X1ml+1X5ml