Benzamidine Beads 4FF

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1. Product Description

Benzamidine Beads 4FF is an affinity medium for purification of trypsin and trypsin-like serine protease, as well as, zymogens including urokinase and prekallikrein. P-Aminobenzamidine, a synthetic inhibitor of trypsin and trypsin-like serine proteases, is covalently coupled via an amide bond to a long spacer arm attached to high cross-linked 4% beads via a stable ether linkage. **Benzamidine Beads 4FF** can easily remove or purify serine proteases from serum, monoclonal cell supernatants and bacterial lysates. The characteristics of **Benzamidine Beads 4FF** are summarized in table 1.

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

Item	Description
Matrix	Highly cross-linked 4% agarose
Ligand	P-Aminobenzamidine
Static binding capacity	>20mg/ml medium
Particle size (μm)	45-165
Maxi pressure	0.3 MPa, 3 bar
pH stability	2-8
Srorage buffer	20% ethanol in 0.05M acetate buffer, pH4.0
Storage temperature	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 to filter the buffers by passing them through a 0.22μm or 0.45 μm filter before use.

Binding Buffer: 50mM Tris-HCl, 0.5M NaCl, pH7.4

Elution Buffer: 10mM HCl, 0.5M NaCl, pH2.0 or 50mM glycin, pH3.0

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

Competitive elution Buffer: 20mM P-Aminobenzamidine in binding buffer

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

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, **Benzamidine Beads 4FF** 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 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

Benzamidine Beads 4FF is stable at pH2-8. Short term of pH1-9 can be used, since more than half of the trypsin binding capacity remains after 1week contact time at pH9. However, prolonged exposure to pH below 2 and above 8 should be avoided due to a slow decomposition of the matrix at low pH and hydrolysis of the ligand.
A general recommendation is to use a guanidine hydrochloride solution to remove precipitated or denatured substances. For hydrophobically bound substances, a solution of nonionic detergent or ethanol is recommended.
For sanitization of **Benzamidine Beads 4FF**, storage in 0.1M acetic acid and 20% ethanol is recommended.

4. Related Products

Product	Cat. No.	Size
Benzamidine Beads 4FF	SA044005	5ml
	SA044025	25ml
	SA044100	100ml
	SA044500	500ml
	SA04401L	1L
	SA04410L	10L
PreCap Benzamidine 4FF	SA044C11	1×1 ml
	SA044C51	5×1 ml
	SA044C15	1×5 ml
	SA044C55	5×5 ml
	SA044CS	3×1 ml+1×5 ml