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Plasmid Bind Beads 6FF

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1. Product Description

Plasmid Bind Beads 6FF is a thiophilic aromatic chromatography medium. It selectively separates covalently closed circular superhelical plasmid DNA from open circular structures. Plasmid Bind Beads 6FF is based on a highly crosslinked 6% agarose gel chemically coupled with mercapto-pyridine ligands that can withstand pressures up to 0.3 MPa. The hydrophilic nature of this matrix ensures low levels of non-specific binding. Plasmid Bind Beads 6FF guarantees that the purification results are consistent from research scale to cGMP production.

Table 1. Characteristics of **Plasmid Bind Beads 6FF**

Item	Description
Matrix	High cross-linked 6% agarose beads
Ligand	Pyridine
Ligand density	~2 mg sc pDNA/ml medium
Particle size (μm)	45-165
Maxi pressure	0.3 MPa，3 bar
pH stability	3-11
Storage buffer	1X PBS containing 20% ethanol

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: 100 mM Tris，2.3 M $(NH_4)_2SO_4$，10 mM EDTA，pH 7.5

Wash Buffer: 100 mM Tris，2.0 M $(NH_4)_2SO_4$，10 mM EDTA，pH 7.5

Elution Buffer: 100 mM Tris，0.3 M NaCl，1.7 M $(NH_4)_2SO_4$，10 mM EDTA，pH 7.5

Sample diluent： 200 mM Tris，4.6 M $(NH_4)_2SO_4$，20 mM EDTA，pH 7.5

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, **Plasmid Bind 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 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

In general, **Plasmid Bind Beads 6FF** is well suited for reuse several times. When reduced performance or an increase in back-pressure are noted, you need to clean the medium with the solutions as follows

- 3 column volumes of deionized water;
- 3 -5column volumes of 0.5 M NaOH solution (contact time usually 15-30minutes);
- Re-equilibrate the medium with deionized water until the effluent is at neutral pH.

4. Related Products

Product	Cat. No.	Size
Plasmid Bind Bead 6FF	SA066005	5 ml
	SA066025	25 ml
	SA066100	100 ml
	SA066500	500 ml
	SA06601L	1 L
	SA06610L	10 L