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Butyl Beads 4FF
Octyl Beads 4FF
Phenyl Beads 6FF(Low Sub)
Phenyl Beads 6FF(High Sub)

Index

1. Product Description

Butyl Beads 4FF, Octyl Beads 4FF, Phenyl Beads 6FF(Low Sub) and Phenyl Beads 6FF(High Sub) are media for Hydrophobic Interaction Chromatography(HIC). Substances are separated on the basis of their varying strength of their hydrophobic interaction with hydrophobic groups attached to the uncharged matrix. They are developed and supported for process scale chromatography. The base matrix of **Butyl Beads 4FF, Octyl Beads 4FF, Phenyl Beads 6FF(Low Sub) and Phenyl Beads 6FF(High Sub)** is highly cross-linked agarose. They have high chemical and physical stability. Details see table under each respective HIC media.

Butyl Beads 4FF

Butyl Beads 4FF is aliphatic hydrophobic interaction medium. The butyl group is coupled to beads by ether linkage, giving a hydrophobic medium with minimal leakage and no ionic properties.

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

Item	Description
Matrix	Highly cross-linked 4% agarose
Type of ligand	Butyl
Capacity (/ml mediun)	approx.7 mg IgG，＞26 mg BSA
Particle Size (μm)	45-165
Flow rate	300 cm/h
pH stability	3-13
Storage buffer	20% ethanol
Storage	4℃ - 30℃

Octyl Beads 4FF

Octyl Beads 4FF is aliphatic hydrophobic Interaction medium. The octyl group is coupled to beads by ether linkage, giving a hydrophobic medium with minimal leakage and no ionic properties.

Table 2. Characteristics of **Octyl Beads 4FF**

Item	Description
Matrix	Highly cross-linked 4% agarose
Type of ligand	Octyl
Capacity (/ml medium)	approx.26 mg IgG, ＞7 mg BSA
Particle Size (μm)	45-165
Flow rate	300 cm/h
pH stability	3-13
Storage buffer	20% ethanol
Storage	4℃ - 30℃

Phenyl Beads 6FF(Low Sub) and **Phenyl Beads 6FF(High Sub)** consist of 90 μm beads of 6% highly cross-linked agarose. The phenyl group is coupled to beads by ether linkage, giving a hydrophobic medium with minimal leakage and no ionic properties. According to the required separation selectivity, efficiency and binding capacity.Different substituted medium are available.

Table 3. Characteristics of **Phenyl Beads 6FF(Low Sub)** and **Phenyl Beads 6FF(High Sub)**

Item	Description
Matrix	Highly cross-linked 6% agarose
Type of ligand	Phenyl
Capacity (/ml medium)	Low Sub:approx.10 mg IgG, ＞24 mg BSA High Sub:approx.30 mg IgG, ＞36 mg BSA
Particle Size (μm)	45-165
Flow rate	300-600 cm/h
pH stability	3-13
Storage buffer	20% ethanol
Storage	4℃ - 30℃

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 0.45μm filter before use.

Binding/wash buffer: 0.05 M phosphate, 1.7 M $(NH_4)_2SO_4$, pH7.0

Elution Buffer: 0.05 M phosphate, pH7.0

Note: The buffer of HIC can be changed according to different samples and the medium.The salt concentration of buffer is high in Binding/wash buffer and low in elution buffer.

2.2 Sample Preparation

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

The salt concentration in the sample is the same as binding /wash buffer. It is usually 0.5-2.0 M $(NH_4)_2SO_4$.

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, the resin 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) Maintain packing flow velocity for at least 3 bed volumes. When the bed has stabilized, mark the bed height on the column and 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 into the column until it reaches the mark, allowing the packing solution to flush the adapter inlet. Lock the adapter in position.

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.

Note:Hydrophobic interaction is weaker at lower temperatures. This must be taken into account if chromatography is done in a cold room.

4) Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

5) Elute with Elution Buffer using a stepwise or linear gradient. For one-step elution, 5 column volumes are usually enough. Other volumes may be required if the interaction is difficult to break. Linear gradient elution can be used to separate proteins of different binding strengths with a small gradient, such as 20 column volumes or more.

2.5 Analysis

Identify the fractions containing the target protein. using UV absorbance, SDS-PAGE, or western blot.

3. Clean-in-Place

After each separation, elute reversibly bound material with low ionic strength buffer. Wash the column wit 5 column volumes of distilled water and 30%isopropanol.

- **Remove strongly hydrophobically bound proteins, lipoproteins and lipids**

Wash the column with 3 column volumes of 70% ethanol or 30% isopropanol (apply increasing concentration gradients to avoid air bubbles formation) . Alternatively, wash the column with 3 column volumes of 0.1-0.5% detergent in a basic or acidic solution. For example, wash with 0.1-0.5% non-ionic detergent in 0.1 M acetic acid .Contact time 1-2 h.

Wash the column with distilled water and re-equilibrate.

- **Sanitization reduces microbial contamination**

Wash the column with 1 M NaOH. Contact time 30-60 min.

Wash the column with distilled water and re-equilibrate.

4. Related Products

Product	Cat. No.	Size
Butyl Beads 4FF	SH001025	25 ml
	SH001100	100 ml
	SH001500	500 ml
	SH00101L	1 L
Octyl Beads 4FF	SH003025	25 ml
	SH003100	100 ml
	SH003500	500 ml
	SH00301L	1 L
Phenyl Beads 6FF (Low Sub)	SH004025	25 ml
	SH004100	100 ml
	SH004500	500 ml
	SH00401L	1 L
Phenyl Beads 6FF (High Sub)	SH006025	25 ml
	SH006100	100 ml
	SH006500	500 ml
	SH00601L	1 L

PreCap Butyl 4FF	SH001C11	1X1 ml
	SH001C51	5X1 ml
	SH001C15	1X5 ml
	SH001C55	5X5 ml
PreCap Octyl 4FF	SH003C11	1X1 ml
	SH003C51	5X1 ml
	SH003C15	1X5 ml
	SH003C55	5X5 ml
PreCap Phenyl LS 6FF	SH004C11	1X1 ml
	SH004C51	5X1 ml
	SH004C15	1X5 ml
	SH004C55	5X5 ml
PreCap Phenyl HS 6FF	SH006C11	1X1 ml
	SH006C51	5X1 ml
	SH006C15	1X5 ml
	SH006C55	5X5 ml
PreCap Select	SH009CS	4X1 ml