BESTCHROM
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Bestarose 4FF
Bestarose 6FF
Agarose Resin
Instruction for use

Contents

1. Introduction

Bestarose 4FF(Fast Flow)and Bestarose 6FF are gel filtration resin made by emulsification, rinsing and sieving of 4% and 6%，respectively. They are used for separation, purification, or detection of biological macromolecules such as polysaccharides, nucleic acids and viruses. This resin is often used as a matrix for affinity, hydrophobic and ion exchange chromatography. Bestarose 4FF and Bestarose 6FF are crosslinked with bifocal crosslinkers based on the gels of Bestarose 4B and Bestarose 6B, and the degree of crosslinking is higher than that of Bestarose CL-4B and Bestarose CL-6B.More stable physical and chemical properties, this kind of resin can withstand 121°C autoclave, fast flow velocity, suitable for large-scale separation and purification applications.

2.Technical characteristics

Product		Bestarose 4FF	Bestarose 6FF
Appearance		White slurry, can be layered	
Agarose concentration		4%	6%
Separation range	Linear numerator	30KD~5000KD	10KD~1000KD
	Globular molecule	60KD~20000KD	10KD~4000KD
Particle size +		45-165μm	
Average particle size		90μm	
Pressure flow velocity++		200-300cm/h（0.1MPa BXK50/60，H=25cm，20°C，in water）	≥200cm/h（0.1MPa BXK50/60，H=25cm，20°C，in water）
Max. pressure		0.3MPa(3bar)	
pH stability		2~12(working),2~14(CIP)	
Chemical stability		Stable in common aqueous buffers: 0.5M NaOH+++,2M NaOH+++,6M GuHCl,8M Urea, 1M acetic acid, 30% acetonitrile	Stable in common aqueous buffers: 0.5M NaOH+++,2M NaOH+++,1M acetic acid, 30% acetonitrile, 8M Urea, 6M GuHCl
Sterilization		Can tolerate 121℃ high pressure sterilization (20min)	
Storage++++		2~30℃, 20% ethanol or 2% benzyl alcohol	

+Particle size is normally distributed, and particles within this range account for more than 95% of the total.

++ The pressure flow velocity describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin

+++0.5M NaOH and 2M NaOH only be used for cleaning.

++++ 2% benzyl alcohol is only used for international transport or special requirements from customer

3. Method of chromatographic

3.1 Column packing

Note：It is best to equilibrate the resin slurry to room temperature before column packing.

- According the column volume to calculate the amount of resin.

  Resin volume=column volume×1.15(Compression factor=1.15)

  According to the volume of the settlement resin required, the suspended slurry of the resin required is calculated by the follow:

  Required resin slurry[1] volume = Settlement resin volume ÷ Resin slurry [1] concentration. The original concentration of resin slurry [1] is shown in the follow table.

Pack size	Resin slurry [1] concentration (%)
100mL、500mL、1L、5L、10L	80
20L、40L	75

1: It refers to the original packaging resin slurry sold by Bestchrom.

Note:For non-original packaging, customer can calculate the required volume according to the actual concentration of resin slurry.

- Washing the resin: Thoroughly shake the resin and weigh certain volume of resin calculated by the method mentioned above. Pour it into a funnel, drain the liquid, and wash with about 3mL distilled water/mL resin for 3 times. Use a glass stick or stirrer to stir each time when adding distilled water, which helps to wash the shipping solvent away.
- Prepare the packing slurry: Transfer the washed resin from the funnel into a beaker or other appropriate container, add distilled water to obtain a 50%~75% slurry, stir well and set aside for use.
- Take a cleaned BXK column (BXK series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications).Take BXK16/70 for example, purge the bubbles trapped at the end-piece net by draining some distilled water through the column outlet. Leave about 1cm water at the bottom of the column and close the bottom outlet. Adjust the column so that it is perpendicular to the ground.
- Slowly pour the slurry into the column at one time (use a packing reservoir if necessary). Do not bring any air bubbles into the column.

  Packing reservoir: Empty glasstube with same diameter as the BXK column.
- Fill the remainder of the column with packing solution. Connect the packing reservoir to the chromatography system, open the flow velocity, drain the bubbles in the hose, close the flow velocity, and tighten the top cover of the packing reservoir.

✧ After pouring, stir well again with Stirrer, and then wash the resin particles on the inner wall of the column from top to bottom with the packing solution, and let the resin settle naturally until there is about 1cm of clarifying solution on the suspension. Mount the adapter and connect the adapter to the chromatography system or peristaltic pump. Lower the adapter to descend to

contact with the clarifying solution and tighten the sealing ring after it is fully immersed in the clarifying solution. With the outlet of the top piece is opened, slowly move the adapter down until all bubbles are drained.

Note: This operation is only applicable to BXK 100 and above columns. Flushing the inner wall reduces the resin particles sticking between the seal ring and the column wall, avoiding the risk of leakage.

- When the bed height is 60cm, the flow velocity can be set to 30cm/h. Open the bottom plug, start the pump and run the setting flow velocity until the bed is stabilized, mark the bed height.
- Remove the packing reservoir (if any), Install the adaptor lower the adaptor to about 0.5cm above the resin surface, set the flow velocity (Bestarose 4FF 150cm/h, Bestarose 6FF 480cm/h), and continue to press the column using the above flow velocity until the bed is completely consolidated, mark the consolidated bed height.
- Stop the pump, open top plug, close the bottom plug, loosen the O-ring seal slightly, press the adaptor to about 0.3cm below the marked position, tighten the O-ring seal, close adaptor stop plug, and complete the column packing.

3.2 Evaluation of Packing

- The packing quality of chromatographic column can be confirmed by column efficiency measurement and evaluation.The tests are required after the column packing, during the column working life and when the separation and purification performance weakens. The method usually relies on the height equivalent to a theoretical plate(HETP) and the asymmetry factor(As).
- Acetone or NaCl solution can be used as sample for the testing. Sample solution and mobile phase can be prepared according to the following table.

	Acetone method	NaCl method
Sample	1.0%(v/v)acetone in water	0.8M NaCl in water
Sample volume	1.0%CV	1.0%CV
Mobile phase	Water	0.4M NaCl in water
Flow velocity	20cm/h	20cm/h
Monitor	UV280 nm	Conductivity

- Method for measuring HETP and As:

  Use UV curve or the conductivity curve to calculate the height equivalent of theoretical plate (HETP), number of theoretical plates(N) and the asymmetry (As):

HETP=L/N

$N=5.54(V_R/W_h)^2$

Note: V_R = retention volume

W_h = half-peak width

L = column height

N = the number of theoretical plates

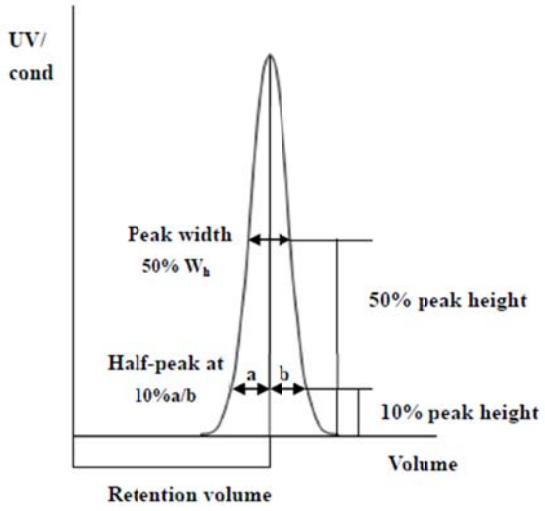
(The units of V_R and W_h should be the same)

As=b/a

Note:

a= 1st half peak width at 10% of peak height

b= 2nd half peak width at 10% of peak height



- Evaluation the column packing

  As a guideline, if the value of HETP is less than 3 times the average particle size(d_{50}) of the resin and the As is between 0.8~1.5, the column is very efficient. The unsatisfactory results should be analyzed and the column should be repacked.

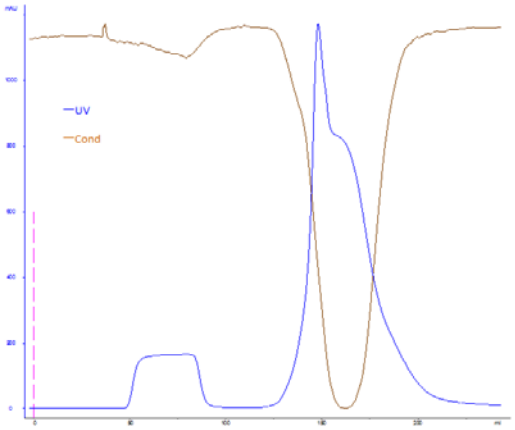
3.3 Chromatographic method

- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45μm before loading, the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer (the pH and conductivity of the sample can be adjusted by dilution, ultrafiltration, and desalination with Bestdex G-25).
- Balancing: Use the recommended flow velocity to rinse the chromatography column with equilibration buffer. The choice of equilibration buffer depends on the stability of the sample. The type and pH of the buffer solution have little effect on the gel filtration effect, but the agarose contains a small amount of sulfate and carboxyl groups. To reduce the non-specific adsorption of basic protein sample, it is recommended to add at least 0.15M NaCl to the equilibrium. The pH and conductivity of the buffer to be exported are consistent with the buffer before entering the chromatography column, which means that the chromatography column is well balanced, generally2~5CV volumes are required.
- Sampling: The sample is loaded onto the chromatography column by means of the loading ring and other devices of the chromatography system. The volume of the loading sample will be different according to the difference of the object and impurities. Generally, the volume of the loading sample is 0.5~5% of the column volume, and the volume of the loading sample can be adjusted appropriately according to the separation effect.
- Separation: Continue to rinse the chromatography column with equilibration buffer to collect the different components that flow out, until no more biomolecules flow out, generally1~1.5CV are required.
- Regeneration: 2~3CV volumes of the chromatographic column were washed with a balanced buffer solution.

- Rebalancing: After rinsing with equilibration buffer, the second sample can be loaded and repeated.

4. Application

Bestarose 6FF removes RNA in DNA purification



Column： BXK26/40
Bed height： 28cm
Buffer： 2.1 M $(NH_4)_2SO_4$
10 mM EDTA+100 mM Tris pH7.50
Sample load：40mL
Flow velocity： 8mL/min

5. Cleaning-in-place(CIP)

In some processes of Bestarose 4FF and Bestarose 6FF, denaturated proteins, lipids, and strong hydrophobic proteins cannot elute in the regeneration process, or after a period of use, column efficiency may decrease, back pressure may increase, separation effect may deteriorate, and color of chromatography resin changes, etc. The following process can be used for in-place cleaning (CIP).

- 1CV of 1M NaCl was used to wash out the proteins with relatively tight binding.
- Removal of strong hydrophobic proteins and precipitating proteins: Clean with 0.5M NaOH of 1CV first, then rinse immediately with 5~10CV pure water.
- Removal of lipoproteins and lipids: Clean with 70% ethanol or 30% isopropanol by volume of 2~3CV first, then rinse with pure water by volume of 5~10CV.

Note: 70% ethanol or 30% isopropanol should be degassed before use. Reverse flushing can be used when the blockage is serious.

6. Sterilization

Since 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that the Bestarose 4FF and Bestarose 6FF resin can be treated with 0.5~1M NaOH to reduce the risk of microbial contamination before and during use. It can also be autoclaved at 121℃ for 20min.

7. Storage

Bestarose 4FF and Bestarose 6FF are supplied in 20% ethanol or 2% benzyl alcohol. They should be stored in 20% ethanol and sealed at 2-30℃ after use, in order to prevent ethanol volatilization and microbial growth, it is recommended to replace the storage solution every 3 months.

8. Disposal and Recycling

Bestarose 4FF and Bestarose 6FF are very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Code No.	Pack size
Bestarose 4FF	AG0052	100mL
	AG0053	500mL
	AG0054	1L
	AG0055	5L
	AG0056	10L
	AG2157	20L
	AG214316	40L
Bestarose 6FF	AG0062	100mL
	AG0063	500mL
	AG0064	1L
	AG0065	5L
	AG0066	10L
	AG2167	20L
	AG215316	40L

Prepacked columns	Code No.	Pack size
EzLoad 16/60 Bestarose 4FF	EG214301	1 pcs
EzLoad 26/60 Bestarose 4FF	EG214302	1 pcs
EzLoad 16/90 Bestarose 4FF	EG027	1 pcs
EzLoad 26/90 Bestarose 4FF	EG00513	1 pcs
EzLoad 16/60 Bestarose 6FF	EG215301	1 pcs
EzLoad 26/60 Bestarose 6FF	EG215302	1 pcs
EzLoad 16/90 Bestarose 6FF	EG028	1 pcs
EzLoad 26/90 Bestarose 6FF	EG00613	1 pcs