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

Contents

1. Introduction

Bestarose 4B and Bestarose 6B 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. The gel structure of Bestarose 4B and Bestarose 6B is fixed by hydrogen bonding, the matrix is relatively soft, the pressure resistance is poor, and it is not resistant to autoclaving.

2.Technical characteristics

Product		Bestarose 4B	Bestarose 6B
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++		70~140cm/h（Δp/L=15 BXK50/20，H=10cm，20℃，in water）	≥100cm/h（Δp/L=15 BXK50/20，H=10cm，20℃，in water）
Max. pressure		0.16bar	0.2bar
pH stability		4~9(working),4~9(CIP)	
Chemical stability		Stable in common aqueous buffers:4M urea, 70% ethanol	
Sterilization+++		0.5M NaOH ,2h	
Temperature tolerance		Working temperature:2~40℃, Can't freeze	
Storage++++		2~30℃ ,20% ethanol or 2% benzyl alcohol	
Avoid		Freezing may damage the internal structure of the resin	

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

++The pressure/flow characteristics 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

+++This operation does not guarantee product stability

++++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 BXK16/20 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.

  Note: This operation is only applicable to BXK 50 and below chromatographic columns.

✧ 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 4B, Bestarose 6B can be set to 60cm/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	30cm/h	30cm/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

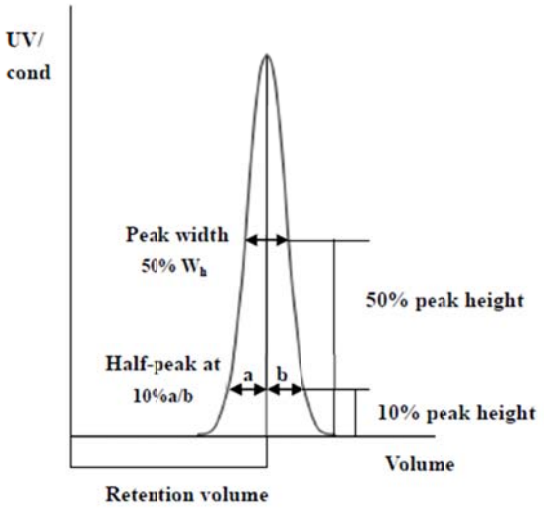
(The units of V_R and W_h should be the same)

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

- Chromatography column pretreatment: The column can be treated with 0.1~0.5M NaOH for more than 2 hours to achieve the purpose of cleaning, disinfection and heat source removal.
- 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).
- Equilibrium: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, generally 2~5CV are required.
- Sampling: The sample is loaded onto the chromatography column through the sample loop of the chromatography system. The volume of the sample will be different according to the difference between the target and impurities. Generally, the sample volume of 2 ~ 5% of the CV is loaded, according to the separation. The effect can be appropriately adjusted with the loading volume.
- Separation：continue to rinse the chromatography column with equilibration buffer to collect the different components that flow out, until no more biomolecules flow out, generally 1~1.5CV are required.
- Regeneration: 2~3CV 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. Cleaning-in-place(CIP)

In some processes of Bestarose 4B and Bestarose 6B,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 medium 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.

5. Sterilization

Since 20% ethanol or 2% benzyl alcohol solution does not have sterilization and depyrogenation, it is recommended that the Bestarose 4B and Bestarose 6B resin can be treated with 0.5M NaOH at the recommended flow velocity to reduce the risk of microbial contamination before and during use.

6. Storage

Bestarose 4B and Bestarose 6B 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.

7. Disposal and Recycling

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

8. Order information

Product	Code No.	Pack size
Bestarose 4B	AG0012	100mL
	AG0013	500mL
	AG0014	1L
	AG0015	5L
	AG0016	10L
	AG209315	20L
	AG209316	40L
Bestarose 6B	AG0022	100mL
	AG0023	500mL
	AG0024	1L
	AG0025	5L
	AG0026	10L
	AG0027	20L
	AG210316	40L