BESTCHROM
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Q XL Big Beads
High load and high flow rate strong anion exchange resin

Instruction for use

Contents

1. Introduction

Ion exchange chromatography(IEC)is a very effective method for separation and purification of biological molecules.The method mainly relies on the interaction between positive and negative charges,and uses the nature and difference of the charges carried by different biomolecules under specific conditions to separate.It has the characteristics of high load capacity, good resolution, controllable conditions and easy amplification. It has been widely used in medicine, chemical industry, metallurgy, food and other fields.

The ion exchange resin is composed of three parts：(1)Cross-linked mesh base frame, which is porous, hydrophilic and chemically stable；(2)The functional group fixed on the base frame, which is a charged group, determines the nature of the ion exchange chromatography resin；(3) An ion (called an equilibrium ion) that has an opposite charge to the functional group and can be reversibly bound to the functional group.

XL Big Beads series resin is to couple linear dextran molecules on the large particle highly cross-linked agarose base, and then couple various functional groups to the dextran molecules. This structure reduces the steric hindrance between biomolecules and can greatly increase the loading of target molecules. The large particle base can tolerate samples with poor clarity, it is not easy to plug the column, and the flow velocity is fast, suitable for processing large samples in a short time.

Q XL Big Beads is a strong anion exchange resin, which can directly capture biological macromolecules from the feed liquid. It has a high loading capacity and a fast flow velocity.

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	6% highly cross-linked agarose with dextran chain
Functional group	Quaternary amine group
Particle size^{+}	100~300μm
Ionic capacity	230~330 μmol Cl^-/mL packed resin
Max. pressure	0.3 MPa
Chemical Stability	Stable in common aqueous buffers: 1M NaOH++,6M GuHCl, 70% ethanol , 30% Isopropyl alcohol Avoid contact with oxidizing agents
pH stability	2~14(CIP)，2~12(working)
Temperature tolerance	Working temperature:2~40℃, Can't freeze.
Storage+++	2~30℃，20% ethanol or 2% benzyl alcohol

Recommended flow velocity	300~900cm/h

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

++1M 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 (%)
25mL、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). 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.

- Set the flow velocity (When the bed height is 15~20cm, the flow velocity can be up to 1100cm/h).Open the bottom plug, start the pump and run the setting flow velocity until the bed is stabilized. If the pressure exceeds 0.3MPa during the packing process, the flow velocity needs to be reduced appropriately. Mark the bed height.
- Remove the packing reservoir (if any), mount the adaptor, lower the adaptor to about 0.5cm above the resin surface, 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

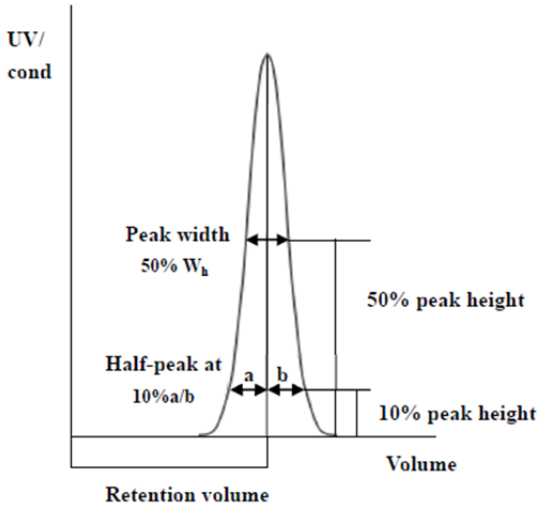
(The units of V_R and W_h should be the same)

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 0.8~1.8, the column is very efficient. The unsatisfactory results should be analyzed and the column should be repacked .

3.3 Chromatographic method

- Buffer selection: Buffer salts whose buffer groups do not act on the resin should be selected. The buffer solution with low salt (less than 5mS/cm) and high pH (usually 1 pH unit higher than the isoelectric point of the target) should be adopted to facilitate the combination of substances. Meanwhile, the stability of samples in the buffer solution should be considered. Elution buffers are usually made by adding a high concentration of salt (e.g. 1M NaCl) or low pH elution to balance buffer.
- Flow velocity: According the column bed high to use the flow velocity 300~900cm/h,the higher column bed high and lower flow velocity.
- Sample preparation: The resin has a certain tolerance to viscosity and granular matter, in order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 5μm 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).
- Equilibration: Washing the column with equilibration buffer until the pH and conductivity of the column outlet buffer are basically the same as the equilibration buffer, which usually needs 3-5CV.
- Sampling: The loading volume is determined according to the substance content in the sample and the binding load of Q XL Big Beads.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution: Linear gradient or step-gradient can be used to increase the elution strength in the elution buffer, eluting substances with different binding strength from the chromatography column,

collecting different components and detecting the location of the target.

- Regeneration: Flush the column with a high concentration of salt (eg: 2M NaCl)
- Rebalancing: After rinsing with equilibration buffer, the second sample can be loaded and repeated.

4. Cleaning-in-place(CIP)

With the increasing use of chromatography resin, the accumulation of contaminants on the chromatography column is also increasing. Cleaning-in-place can prevent the accumulation of contaminants and maintain a stable working state. Determine the frequency of CIP according to the degree of contamination of the resin (if the contamination is serious, CIP should be carried out after each use to ensure repeatability of the results).

The recommended CIP for different types of impurities and contaminants are as follows:

- 2~3CV of 2M NaCl was used to wash out the proteins with relatively tight binding.
- Removal of strong hydrophobic proteins and precipitating proteins: Clean with 1M NaOH of 2~3CV first, then rinse immediately with 5~10CV pure water.
- Removal of lipoproteins and lipids: Clean with 70% ethanol or 30% isopropanol by volume of 5~10CV first, then rinse with pure water by volume of 5~10CV.
- The above two cleaning conditions can also be combined for cleaning, namely 30% isopropanol solution containing 1M NaOH.

  Note: 70% ethanol or 30% isopropanol should be degassed before use. In the CIP process, the flow velocity can be chosen as 30~60cm/h. Reverse flushing can be used when the blockage is serious.

5. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that Q XL Big Beads can be treated with 1M NaOH for more than 0.5-1h to reduce the risk of microbial contamination before and during use.

6. Storage

Q XL Big Beads is supplied in 20% ethanol or 2% benzyl alcohol. It 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

Q XL Big Beads is very difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order information

Product	Code No.	Pack size
Q XL Big Beads	AI0231	25mL
	AI214407	100mL
	AI0233	500mL
	AI0234	1L
	AI0235	5L
	AI0236	10L
	AI214415	20L
	AI214416	40L