BESTCHROM
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BestPoly 30RPC Reversed-phase chromatography resin

Instruction for use

Contents

1. Introduction

BestPoly 30RPC is made of polymerized polystyrene and divinylbenzene, and it is based on the reversed-phase chromatography resin formed by the high-density benzene ring. Compared with the traditional silica-based reversed-phase resin, BestPoly reversed-phase resin can tolerate a higher pH and the particles are more uniform. It has good scalability and is mostly used in the stage of fine purification.

The resin has the following characteristics:

- Uniform particle size distribution
- High resolution
- High pH resistance and low back pressure
- Fast flow velocity
- Stable physical and chemical properties

2.Technical characteristics

Appearance	White to yellowish slurry, can be layered
Matrix	Polystyrene and divinylbenzene particles
Particle size	30μm
Dynamic binding capacity	~ 23mg bacitracin/mL packed resin ~14mg BSA/mL packed resin ~72mg insulin/mL packed resin
Chemical stability	Stable in common aqueous buffers: 1 M NaOH[+],1.0 M HCl, 1.0 M HCl/ 90%methanol,90%acetic acid,6M GuHCl,100%n-propanol,100% ethanol, 100% methanol, 100% acetone, 0.1% TFA (in water), 0.1% TFA (in acetonitrile), 100% isopropanol, 100% tetrahydrofuran.
Max. pressure	10MPa
pH stability	2~12(working) 1~14(CIP)
Temperature tolerance	Working temperature: 2~40℃, Can't freeze, Can tolerate 121℃ high pressure sterilization (20min，pH7)
Storage[++]	2~30℃,20% ethanol or 2% benzyl alcohol

+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

BestPoly 30RPC is stored in 20% ethanol. Before packing the chromatography column, the ethanol solution needs to be replaced with the packing solution and the resin temperature is equilibrated to room temperature.

Due to the fine particles of the resin, it is necessary to choose a chromatography column with a screen below 5 microns and a chromatography column with a relatively high pressure (BXR10 series chromatography columns can be selected during the laboratory process development stage).

- The packing height of BestPoly 30RPC is different according to the type and size of chromatographic column, generally ranging from 0-300mm. It should not be too high.
- According the column volume to calculate the amount of resin.

  Resin volume=column volume×1.1(Compression factor=1.1)

  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: Suspend the resin by shaking and pour into a funnel, remove the liquid, and wash with about 3mL packing solution (25-100% ethanol)/mL resin for 3 times. Use a glass stick or stirrer to stir each time you add the packing solution, in order to better clean the shipping buffer.
- Prepare the packing slurry: Transfer the washed resin from the funnel into a beaker or other appropriate container, and add packing solution to obtain a 25-45% slurry, the slurry concentration should not be too high, otherwise the ideal column efficiency and symmetry will not be achieved. Mix well and set aside.
- Take a cleaned BXK column. Purge the bubbles trapped at the end-piece net by draining some packing solution 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.

- For BXR10/17 chromatographic columns, a pressure of 1.2-1.5MPa is used to install the column, or an appropriate flow velocity is set to achieve a backpressure of 1.2-1.5MPa.After the surface is stable, the cylinder head is lowered to a position of 0.2cm on the surface, and the glue is pressed for 5min according to the flow velocity or pressure above, mark the bed height.
- Stop the pump, open top plug, close the bottom plug, loosen the O-ring seal slightly, press the adaptor to about 0.2cm below the marked position, tighten the O-ring seal, close adaptor stop plug, and complete the column packing.

3.2 Evaluation of Packing

- NaCl can be used as an indicator for column efficiency measurement, and sample solution and mobile phase are prepared according to the table below. Special attention should be paid to reducing the dead volume of the system for the chromatographic column with small column volume to obtain good column efficiency detection results.

	NaCl method
Sample	0.8M NaCl in water
Sample volume	1.0%CV
Mobile phase	0.4M NaCl in water
Flow velocity	60cm/h
Monitor	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

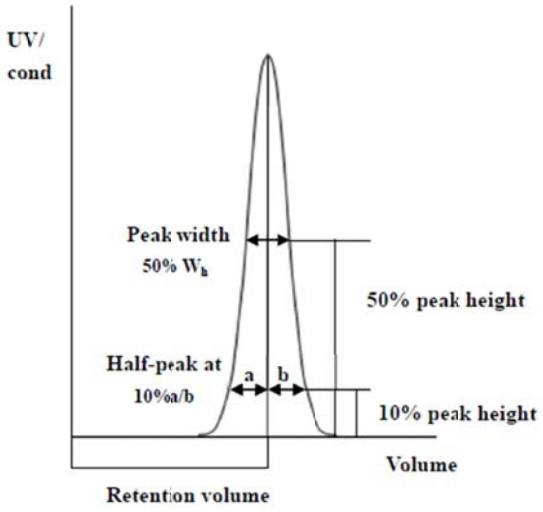
(The units of V_R and W_h should be the same)

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 As is between 0.8~1.8, it is judged to be qualified. The unsatisfactory results should be analyzed and the column should be repacked .

3.3 Chromatographic method

In order to achieve a good purification effect, yield and simple operation, it is necessary to first optimize the process conditions in the laboratory. The main contents of the optimization are:

- Type and concentration of solvent: Generally, the best resolution is obtained when the mobile phase is acetonitrile. Acetonitrile also has low viscosity and good ultraviolet light transmission, but because of its toxicity, its use has limitations. The process scale often uses low alcohols as the first choice. They are relatively cheaper but more viscous, so the back pressure during operation is higher than acetonitrile.

  In the process optimization stage, a solvent gradient test needs to be set to sequentially achieve the required resolution. It usually increases by 5% every hour. Organic solvents with a starting concentration of at least 5% and organic solvents with a maximum concentration not exceeding 95%. Because the use of 0% and 100% organic solvents requires a long equilibration time, and at the same time, the target protein sample has a risk of denaturation and salt precipitation at high solvent concentrations.

- Type and concentration of ionizing agent and solution: TFA is the most widely used ionizing agent, which makes the separation of peptides and proteins better. It is volatile and relatively easy to remove. Triethylammonium phosphate or ethyl acetate (TEAP, TEAA) can be used instead, with different selectivity.

  Other buffer ingredients that can be used are acids, such as phosphoric acid or acetic acid and neutral salts (eg: ammonium sulfate).

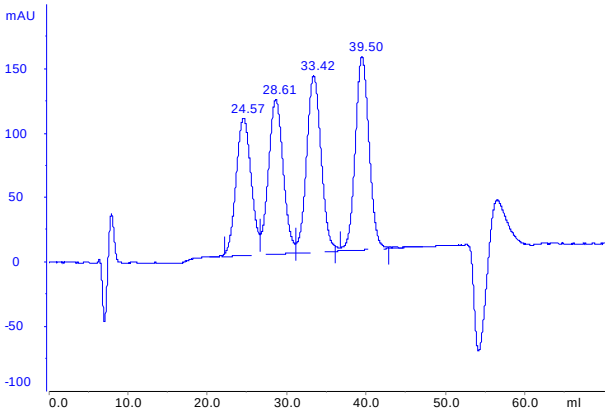
- pH：Different resolutions can be obtained at different pH values. BestPoly 30RPC has good pH stability and can be optimized in the range of 2-12.

  After optimizing the RPC step on a laboratory scale, the method can be scaled up. The enlargement is achieved by increasing the diameter of the chromatography column. Keep the

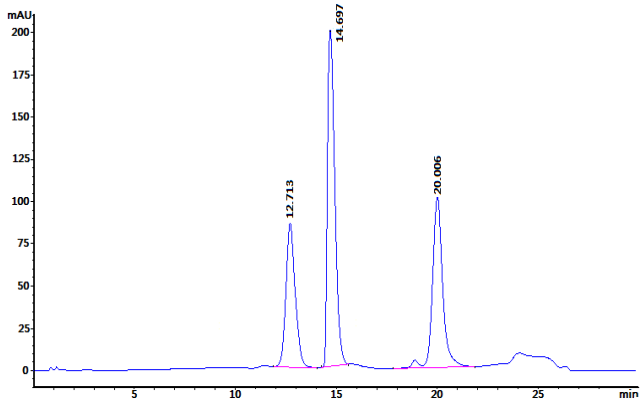
parameters such as column bed height, linear flow velocity, sample concentration and volume constant, and keep the ratio of gradient volume/column bed volume unchanged, so that the diameter and volume flow of the chromatography column will increase.

4、Application

BestPoly 30RPC separates angiotensin and protein isolate



Column：BXR10/17
Mobile phase A: 0.1% trifluoroacetic acid＋water
Mobile phase B: 60.0% acetonitrile, 0.1% trifluoroacetic acid＋water
Samples: Angiotensin I, Angiotensin III, (Val^4) Angiotensin III, (Ile^7) Angiotensin III



Column：BXR10/10
Mobile phase A: 0.1% trifluoroacetic acid＋water
Mobile phase B: 60.0% acetonitrile, 0.1% trifluoroacetic acid＋water
Sample: bovine insulin, BSA, RNase three proteins were dissolved in 20% mobile phase B, the concentration was 5mg / mL.

5. Cleaning-in-place(CIP)

Regular CIP can prevent the accumulation of pollutants, maintain a stable working state, and extend the service life of the resin.

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

- Lipoproteins and lipids removal：Acetonitrile or isopropyl alcohol cleaning.
- Removal of strong hydrophobic proteins and precipitated proteins:1M sodium hydroxide cleaning.
- Acid soluble material removal: 90% acetic acid or 1M hydrochloric acid or 3% trifluoroacetic acid cleaning.

6. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that BestPoly 30RPC can be treated with 0.5~1M NaOH to reduce the risk of microbial contamination before and during use.

7. Storage

BestPoly 30RPC 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.

8. Disposal and Recycling

BestPoly 30RPC is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Code No.	Pack size
BestPoly 30RPC	AR400105	25mL
	AR400107	100mL
	AR400111	500mL
	AR400112	1L
	AR400113	5L
	AR400114	10L