BESTCHROM
博 格 隆

Diamond Butyl Mustang High flow rate and high-resolution hydrophobic chromatography resin

Instruction for use

Contents

1. Introduction

Hydrophobic interaction chromatography (HIC) is a chromatographic method that uses the interaction between the hydrophobic groups carried by biological molecules and the hydrophobic ligands on the stationary phase to separate substances. Salt ions can destroy the hydration membrane on the surface of biomolecules and promote the binding between hydrophobic groups and ligands.

Diamond Butyl Mustang is based on fine-grained high-rigidity agarose with weak hydrophobic aliphatic butyl groups. It has high resolution and low back pressure, and is suitable for the intermediate and fine purification stages of protein downstream purification.

2. Technical characteristics

Appearance	White slurry
Matrix	Highly cross-linked rigid agarose
Functional group	Butyl
Average particle size	36~44μm
Hydrophobicity	The retention time of α - Chymotrypsinogen is 52-58min
Dynamic binding capacity	~37mg BSA/mL packed resin
Max. pressure	0.5MPa
Pressure flow velocity	≥450cm/h(0.5MPa BXK 100/500, H=20cm, 20℃)
Chemical stability	Stable in common aqueous buffers: 1M NaOH+,1M HAc+,6M GuHCl,30% isopropyl alcohol,70% ethanol
pH stability	2~14(CIP),3~13(working)
Operating temperature	Operating temperature: 2~40℃, do not freeze, Can tolerate 121℃ autoclaving (20min)
Storage++	2~30℃, 20% ethanol or 2% benzyl alcohol
Recommended flow velocity	90~180cm/h

+1M NaOH and 1M HAc 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 precipitated resin required, the resin slurry required is calculated by the follow:

  Required resin slurry[1] volume = Precipitated 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 resins in non-original concentration, 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 (20% 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, add packing solution 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 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.

  Description: Purification of biological macromolecules by HIC resin is a typical highly selective technique. The retention of substances to be separated may vary greatly at any particular ionic strength. Therefore, if you want to optimize the selectivity of HIC resin, you can use relatively short columns. Typical bed heights range from 3cm to 15cm to ensure high flow velocity while avoiding excessive backpressure.
- 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 10cm, the flow velocity can be set to 600cm/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), 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 the top plug of adaptor, close the bottom plug, loosen the O-ring seal slightly, press the rubber surface according to the compression ratio of 1.15, tighten the O-ring seal, close the outlet, 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
Detection	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

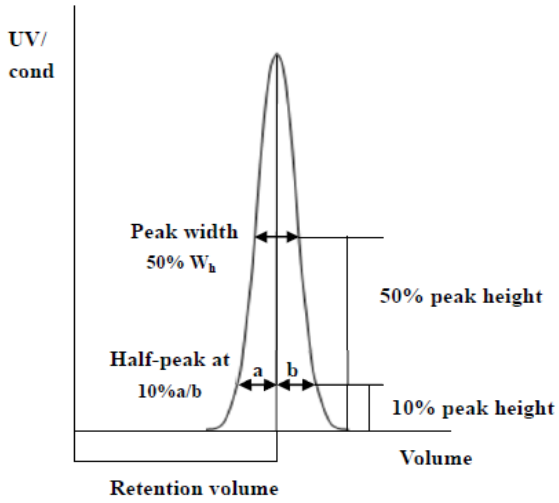
(The units of V_R and W_h should be the same)

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

Note: The temperature has a greater influence on hydrophobic chromatography. Resin hydrophobicity will compromise under low temperature. Keeping the temperature of the environment, buffer and sample consistent during the experiment can ensure the repeatability of hydrophobic chromatography. It is generally controlled at 22~24℃.

- Buffer selection: The binding buffer is usually a phosphate buffer containing a high concentration of salt, such as 20mM PB, 1.5M $(NH_4)_2SO_4$, pH 7.0. The elution buffer usually uses phosphate buffer without other salts, such as 50mM PB, pH 7.0, which needs to be adjusted based on the subsequent experimental results (whether there is precipitation of the target, binding strength of the target, recovery rate, resolution etc.), adjust the concentration and type of salt in the binding buffer. For substances that are difficult to elute, purified water can be used, alternatively, low-concentration ethanol can be added to the purified water as the elution buffer.
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by 0.45μm membrane of 0.45μm before loading, the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer. The loading volume is determined by the impurity content in the sample and the binding capacity of Diamond Butyl Mustang.

  The binding strength of proteins to hydrophobic resin is affected by ligand structure, ligand concentration, ionic strength of buffer, salting out effect (see hofmeister sequence below), temperature, etc.

  Hofmeister sequence:

  ← **Salting out effect increases**

  negative ion:PO_4^{3-},SO_4^{2-},CH_3COO^-,Cl^-,Br^-,NO_3^-,ClO_4^-,I^-,SCN^-

  positive ion:NH_4^+,Rb^+,K^+,Na^+,Cs^+,Li^+,Mg^{2+},Ba^{2+}

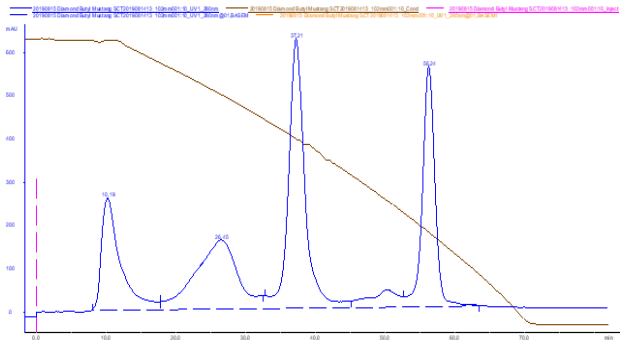
→ **The effect of salt solution increases**

Increasing salting-out effect increases hydrophobic effect, while increasing salting-out effect weakens hydrophobic effect.

- 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.
- Loading Sample: Sample loading shall be carried out according to the set conditions.
- Cleaning: Clean with equilibration buffer until the UV absorption value is close to baseline.
- Elution: A linear gradient or step gradient can be used to increase/decrease the elution intensity in the elution buffer, and substances with different binding strengths can be eluted from the chromatography column to collect different components and detect the location of the target.
- Regeneration: Clean the column with purified water or 30% isopropyl alcohol (70% ethanol).
- Re-equilibration: Clean with equilibration buffer till pH and conductivity reach the same as equilibration buffer, the second sample can be loaded, repeat the process if necessary.

4. Application

Using Diamond Butyl Mustang to separate α-Chymotrypsinogen cytochrome C, lysozyme, and RNase.



Column: BXR 10/17

Equilibration: 20mM Tris 1.7M $(NH_4)_2SO_4$ pH 7.5

Elution: 20mM Tris pH 7.5

Sample: Cytochrome C 1mg/mL
RNA enzyme 2mg/m
Lysozyme 1mg/mL
α -Chymotrypsinogen 1mg/mL

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

- 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.

  Laboratory type BXK small column polyacrylic plastic shell can not tolerate 70% ethanol and other high concentration of organic solvents, pay attention to the use of organic solvents do not spill on the plastic shell.

6. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended to treat Diamond Butyl Mustang with 1M NaOH for 0.5-1h to reduce the risk of microbial contamination before and during use. It can also be autoclaved at 121℃ for 20min.

7. Storage

Diamond Butyl Mustang 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

Diamond Butyl Mustang is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Cat. No.	Pack size
Diamond Butyl Mustang	AH303205	25mL
	AH303207	100mL
	AH303211	500mL
	AH303212	1L
	AH303213	5L
	AH303214	10L
	AH303215	20L
	AH303216	40L

Prepacked columns	Cat. No.	Pack size
EzFast Butyl Mustang	EH01621	1×1mL
	EH303251	5×1mL
	EH303203	1×5mL
	EH303253	5×5mL
EzScreen Butyl Mustang	EH01625	1×4.9mL
	EH01635	5×4.9mL
EzLoad 16/10 Butyl Mustang	EH01601	1 pcs
EzLoad 26/10 Butyl Mustang	EH01611	1 pcs