BESTCHROM
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Plasmid Cap Bestarose HP Affinity chromatography resin

Instruction for use

Contents

1. Introduction

Plasmid Cap Bestarose HP is a thiophilic affinity resin made by fixing sulfur-containing compound 2-mercapyridine on high resolution crosslinked agratose. Its optimized ligand density has appropriate affinity with superhelix DNA, and fine particle microspheres can improve the load of superhelix DNA with larger molecular weight.The principle of thiophilic affinity is to use the interaction between electron donor and electron acceptor to separate and purify biomolecules. This force is strengthened in high salt environment and weakened in low salt environment.

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	Highly Cross-linked agarose, 6%
Average particle size	34μm
Functional group	2-mercaptopyridine
Ligand concentration	~3.5mg 2-mercaptopyridine /mL resin
Dynamic binding capacity	>2mg Supercoiled plasmid DNA /mL packed resin
Chemical Stability	Stable in common aqueous buffers: 1M HAC+ ,30% isopropyl alcohol, 70% ethanol , 0.1M NaOH
Max. pressure	0.3MPa
pH stability	3~11(Working)，2~13(CIP)
Storage++	2~30℃, 20% ethanol or 2% benzyl alcohol
Temperature tolerance	Working temperature:15~30℃

+1M 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 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). 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.

✧ 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 250cm/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 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

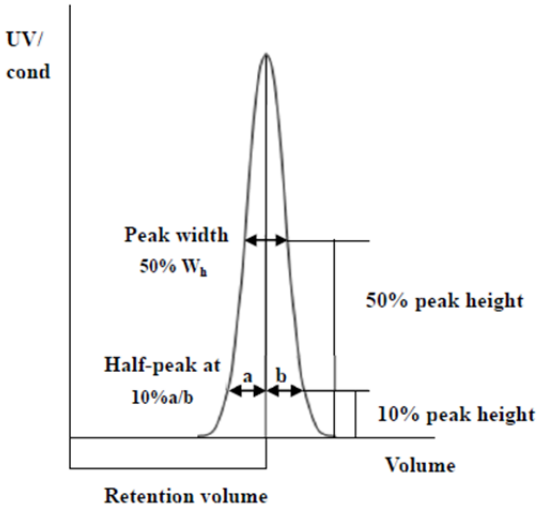
  (The units of V_R and W_h should be the same)

  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

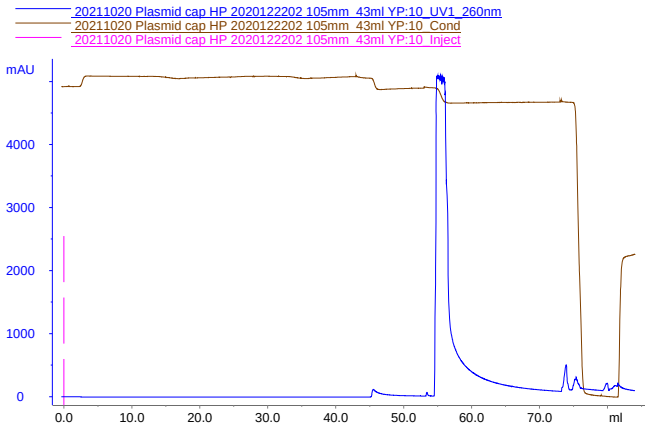
- Recommended buffer:

  Binding buffer: 2.0M（NH_4）$_2SO_4$+10mM EDTA+100mM Tris pH 7.5

  Eluting buffer: 1.7M（NH_4）$_2SO_4$+0.3M NaCl+10mM EDTA+100mM Tris pH 7.5

- Flow velocity: According the column bed high to use the flow velocity 50~120cm/h，the higher column bed high and lower flow velocity.
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45μm before loading.
- Equilibration: Washing the column with equilibration buffer, which usually needs 3-5CV.
- Sampling: The loading volume was determined according to the loading capacity of the resin and the concentration of DNA in the sample.
- Rinse: The plasmid DNA was washed away with the recommended buffer.
- Elution: Elution peaks can be collected using the recommended elution buffer.
- Regeneration: The column was cleaned with 3CV water and then cleaned with 3CV 0.5M NaOH, and the NaOH was cleaned with 3CV water.
- Rebalancing: After rinsing with equilibration buffer, the second sample can be loaded and repeated.

4. Application



Column：BXR5/100, 10cm column height
Sample: A plasmid DNA sample
Binding buffer： 2.1M $(NH_4)_2SO_4$，10mM EDTA
100mM Tris-HCl， PH 7.5
Eluting buffer： 1.7M $(NH_4)_2SO_4$，10mM EDTA
100mM Tris-HCl，0.3M NaCl，PH 7.5
Flow velocity： 1mL/min

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).

- Denatured protein removal: 2~4CV were cleaned with 0.5M NaOH, and the balance buffer with 2~4CV was used after cleaning NaOH with water.
- Strong hydrophobicity or lipid removal: use 2~4CV 20mM PB+30% isopropanol, pH7.5 buffer to clean the column, wash with water before and after.

6. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that Plasmid Cap Bestarose HP can be treated with 70% ethanol for more than 12h to reduce the risk of microbial contamination before and during use.

7. Storage

Plasmid Cap Bestarose HP 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

Plasmid Cap Bestarose HP is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Code No.	Pack size
Plasmid Cap Bestarose HP	AA0201	25mL
	AA116307	100mL
	AA0203	500mL
	AA0204	1L
	AA0205	5L
	AA116314	10L

Prepacked columns	Code No.	Pack size
EzFast Plasmid HP	EA216101	1×1mL
	EA216103	1×5mL
	EA216151	5×1mL
	EA216513	5×5mL
EzScreen Plasmid HP	EA02025	1×4.9mL
	EA02035	5×4.9mL
EzLoad 16/10 Plasmid HP	EA216104	1 pcs
EzLoad 26/10 Plasmid HP	EA216106	1 pcs