BESTCHROM
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IgM Capture Bestarose FF Affinity chromatography resin

Instruction for use

Contents

1. Introduction

IgM Capture Bestarose FF is a sulfur-philic affinity resin made by fixing sulfur-containing compound 2-mercaptopyridine on fine particles of high rigidity agarose. Its optimized ligand density and IgM have suitable affinity, and fine particle microspheres can increase the loading capacity of IgM with larger molecular weight. The principle of sulfur affinity is to use the interaction between the electron donor and the electron acceptor to separate and purify biomolecules. This force is strengthened in a high-salt environment and weakened in a low-salt environment.

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	Highly cross-linked agarose, 6%
Particle size+	45~165μm
Functional group	2-mercaptopyridine
Ligand concentration	~ 2mg 2-mercaptopyridine /mL resin
Chemical stability	Stable in common aqueous buffers:70% ethanol, 30% isopropyl alcohol, 1M HAc++, 0.1M NaOH
Pressure flow velocity	250~400cm/h（0.1MPa BXK50/30 H=25cm 20℃）
Max. pressure	0.3MPa
pH stability	3~11(working)，2~13(CIP)
Storage+++	2~30℃，20% ethanol or 2% benzyl alcohol

+ The particle size is normally distributed, and the particles in this range account for more than 95% of the total

++ 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: Suspend the resin by shaking and pour into a funnel, remove the liquid, and wash with about 3mL packing solution (0.1M NaH_2PO_4 with 1.2M $(NH_4)_2SO_4$, pH7.0)/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.
- 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 75cm/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

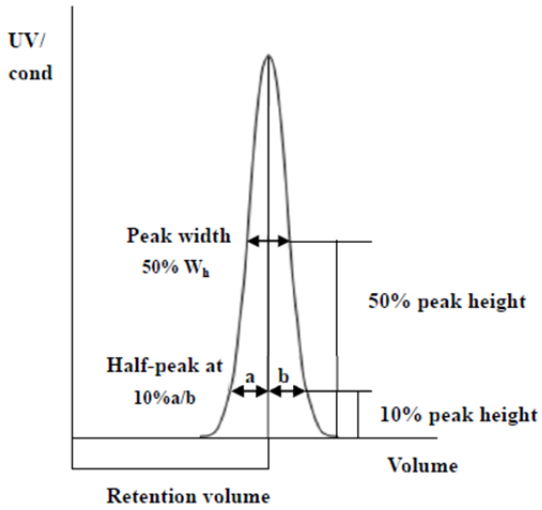
  (The units of V_R and W_h should be the same)

  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

  Binding buffer：20mM PB，0.8M $(NH_4)_2SO_4$，pH7.5（Some monoclonal IgM may not be bound to the column in $(NH_4)_2SO_4$ at 0.8M, at this time, the concentration of $(NH_4)_2SO_4$ can be

increased to 1M to improve the binding capacity.

Elution buffer：20mM PB，pH7.5

Wash buffer：20mM PB，30% isopropanol，pH7.5

In some cases, 0.8M ammonium sulfate can be replaced by 0.5M potassium sulfate. Most monoclonal IgM can bind to the column under 0.5M potassium sulfate. The purity of the purified monoclonal IgM in 0.8M ammonium sulfate and 0.5M potassium sulfate is comparable.

- Flow velocity：According the column bed high to use the flow velocity <150cm/h cm/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, the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer, the concentration of ammonium sulfate affects the binding load.
- 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 IgM Capture Bestarose FF.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution：It can be eluted with the recommended elution buffer. If the target protein is not completely eluted, it can be eluted with 20mM PB pH7.5 buffer containing low concentration (such as 5-10%) of isopropyl alcohol.Note that high concentration of isopropyl alcohol cannot be used for elution.
- Regeneration：Flush the column with washing buffer.
- Re-equilibration：After rinsing with binding 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).

- Denatured protein：Wash 4CV with 0.1M NaOH and immediately rinse with at least 5CV of equilibration buffer.
- Strong hydrophobic substances or lipids：Rinse the column with 2-4CV of 20 mM PB, 30% isopropyl alcohol, pH 7.5 buffer, and immediately flush with at least 5CV of equilibration buffer.

5. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that IgM Capture Bestarose FF can be treated with 70% ethanol for more than 12h to achieve the purpose of sterilization and depyrogenation.

6. Storage

IgM Capture Bestarose FF 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

IgM Capture Bestarose FF is very difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order information

Product	Code No.	Pack size
IgM Capture Bestarose FF	AA0172	25mL
	AA0173	100mL
	AA0174	500mL
	AA0175	1L
	AA0176	5L
	AA0177	10L

Prepacked columns	Code No.	Pack size
EzFast IgM FF	EA214301	1×1mL
	EA042	5×1mL
	EH214303	1×5mL
	EA043	5×5mL
EzScreen IgM FF	EA01725	1×4.9mL
	EA01735	5×4.9mL
EzLoad 16/10 IgM FF	EA214304	1 pcs
EzLoad 26/10 IgM FF	EA214306	1 pcs