BESTCHROM
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# IgM Cap Mustang Affinity chromatography resin

# Instruction for use

# Contents

# 1. Introduction

IgM Cap Mustang 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, while ist 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 |
| Matrix | Highly cross-linked agarose, 6% |
| Average particle size + | 36~44μm |
| Functional group | 2-mercaptopyridine |
| Ligand concentration | ~ 2mg 2-mercaptopyridine /mL resin |
| Dynamic binding capacity | 5mg human IgM/mL resin |
| Chemical stability | Stable in common aqueous buffers:70% ethanol,30% isopropyl alcohol,1M HAc++,0.1M NaOH |
| Max. pressure | 0.5MPa |
| pH stability | 2~13(CIP),3~11(working) |
| Storage+++ | 2~30℃,20% ethanol or 2% benzyl alcohol |

+ Average particle size is the accumulated resin particle size of packing volume distribution.

++ 1M HAc only be used for cleaning.

+++2% benzyl alcohol is only used for international transport or special requirements from customer.

# 3. Method of chromatography

## 3.1 Column packing

**Note:It is best to equilibrate the resin slurry to room temperature before column packing. Due to the fine particle size of the resin, a chromatography column with a mesh size of 10 microns or less should be selected.**

- 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 suspended resin required, the suspended slurry of the resin required is calculated by the follow:

  Required resin slurry[1] volume = Suspended 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 (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 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 |
| 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

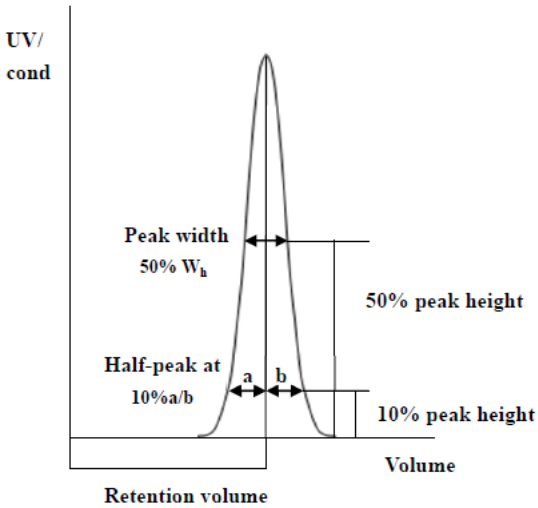
  (The units of $V_R$ and $W_h$ should be the same)

  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

- 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.

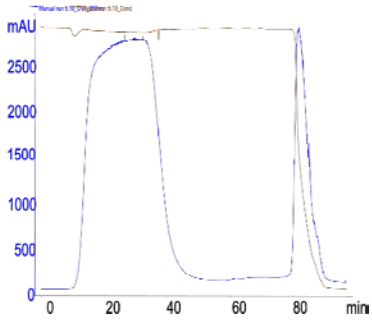
  Elution buffer: 20mM PB, pH7.5

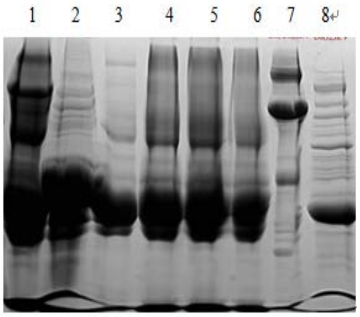
  Cleaning buffer: 20mM PB, 30% isopropanol, pH7.5

  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 height to use the flow velocity (usually lower than <150cm/h cm/h), the higher column bed height is, the lower flow velocity will be.
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by 0.45μm microporous membrane 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 capacity.
- 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: The loading volume is determined by the substance content in the sample and the binding capacity of IgM Cap Mustang.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is close to baseline.
- 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: Wash the column with cleaning buffer.
- Re-equilibration: After rinsing with binding buffer, the second sample can be loaded, repeat the process if necessary.

## 4. Application

IgM Cap Mustang purified mouse IgG chromatogram




Electrophresis sequence (reduction:DDT reduction)
Lane1: Sample
Lane2: Sample (reduction)
Lane3: Flow through
Lane4: Flow through
Lane5: Flow through

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

## 6. Sterilization

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

## 7. Storage

IgM Cap 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 regularly.

## 8. Disposal and Recycling

IgM Cap Mustang is very difficult to degrade in nature, incineration is recommended to protect the environment.

## 9. Order information

| Product | Cat. No. | Pack size |
|---|---|---|
| IgM Cap Mustang | AA0162 | 25mL |
| | AA0163 | 100mL |
| | AA0164 | 500mL |
| | AA0165 | 1L |
| | AA0166 | 5L |
| | AA314214 | 10L |

| Prepacked columns | Cat. No. | Pack size |
|---|---|---|
| EzFast IgM Mustang | EA314301 | 1×1mL |
| | EA314303 | 1×5mL |
| | EA039 | 5×1mL |
| | EA040 | 5×5mL |
| EzScreen IgM Mustang | EA01625 | 1×4.9mL |
| EzScreen IgM Mustang | EA01635 | 5×4.9mL |
| EzLoad 16/10 IgM Mustang | EA314304 | 1 pcs |
| EzLoad 26/10 IgM Mustang | EA314306 | 1 pcs |