BESTCHROM
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Extrem A Diamond Affinity chromatography resin

Instruction for use

Contents

1. Introduction

Extrem A Diamond is an innovative enhanced alkali-resistant resin independently developed by Bestchrom. The protein A ligand is expressed in E. coli. Compared with previous AT Protein A Diamond Plus, it enjoys higher tolerance towards alkali (can tolerate CIP with 0.5-1.0M NaOH) and higher binding capacity. It can effectively cut cost during scale-up by choosing lower column volume. Besides, It is suitable for capturing monoclonal antibodies or Fc fusion protein from large volume cell culture media, as well as the capture of polyclonal antibodies from ascites or plasma.

The resin has the following characteristics:

- Under the same residence time, it has higher dynamic binding capacity than conventional products.
- The high alkaline tolerance of protein ligands can withstand CIP of 0.5~1.0M NaOH during the cleaning process.
- High binding capacity, reduced production cost and improved production efficiency.

2. Technical characteristics

Appearance	White slurry
Matrix	High rigidity agarose
Average particle size +	~60μm
Functional group	Recombinant Alkali Tolerant Protein A
Cross-linking method	Epoxy chemistry
Dynamic binding capacity ++	≥75mg Human IgG/mL packed resin(Residence time 6min)
Chemical stability	Stable in common aqueous buffers: 10mM HCl, 0.1Msodium citrate, 6M GuHCl, 8M Urea, 30% Isopropyl alcohol, 20% ethanol.
Max. pressure	0.5MPa
Pressure flow velocity	≥300cm/h,<2bar,BXK300/500,H=20cm
pH stability	3~12(working) 2~14(CIP)
Storage+++	2~8℃, 20% ethanol or 2% benzyl alcohol

+Particle size is normally distributed, and particles within this range account for more than 75% of the total.

++Linear flow velocity 100cm / h, column height 10cm, buffer conditions: 20mM PB, 0.15M NaCl, pH7.4.

+++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.

- According the column volume to calculate the amount of resin.

  Resin volume=column volume×1.18(Compression factor=1.18)

  According to the volume of the precipitated resin required, the resin slurry required is calculated by the follow:

  Required resin slurry1 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 with 0.4M NaCl)/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 45%~55% 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 300cm/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 resin surface according to the compression ratio of 1.18, 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

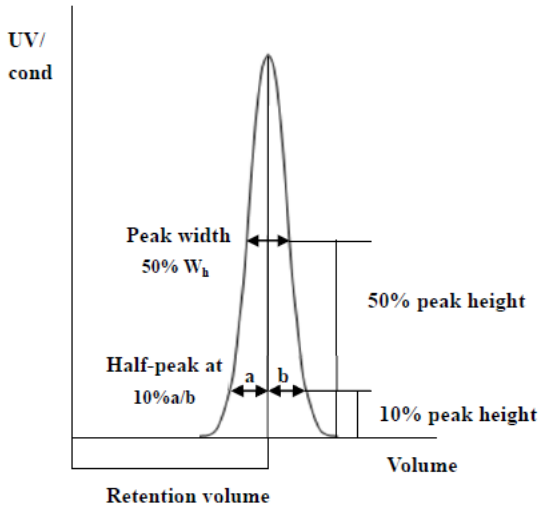
  (The units of V_R and W_h should be the same)

  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

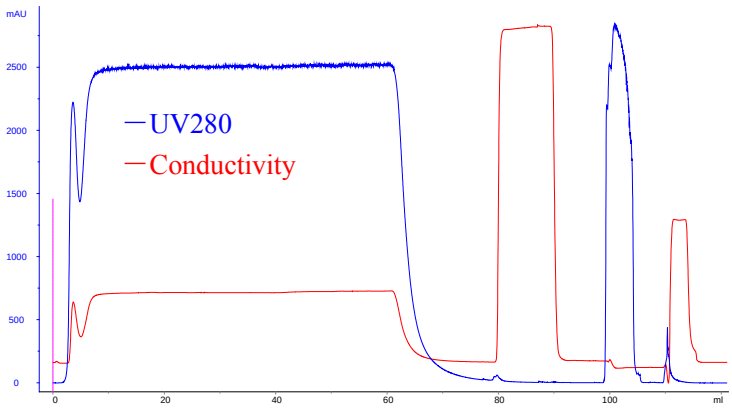
- Buffer: Usually use neutral buffer as the binding buffer (e.g. 50mM PB 0.15M NaCl pH7.0-7.6). Use low pH buffer as elution buffer (e.g. 0.1M citric acid pH3.0-4.0). Since the binding ability of AT Protein A to IgG depends on the source and subtype of antibody (Table 1), high salt and high pH can promote the binding of antibody and resin, reduce non-specific binding, increase pH, as well as neutralize the relative histidine residues of the alkali-resistant Protein A and IgG binding sites. The electrostatic repulsion effect of these residues hinders the affinity reaction. So, increase the salt concentration to reduce electrostatic repulsion and enhance the hydrophobic effect. For different antibodies, the binding conditions and washing conditions can be optimized by changing the salt type, concentration and pH of the buffer.

  When optimizing the elution conditions, it is necessary to explore the maximum pH for effective desorption. Avoid low pH due to the denaturation of unstable antibodies under that condition.
- Flow velocity: According to the height of the column, a linear flow velocity of 60 ~ 300cm / h is generally selected. The higher the column height, the slower the flow velocity.
- Equilibration: Washing the column with equilibration buffer, which usually needs 3-5 CV.
- Sample preparation: In order to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45μm microporous membrane before loading, and the pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer.
- Sample loading: The loading volume is determined by the substance content and binding capacity of Extrem A Diamond.

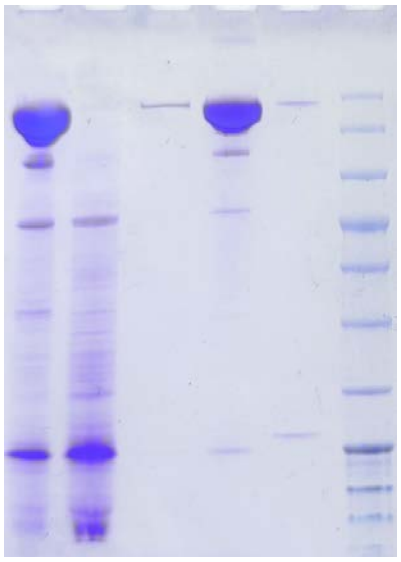
- Cleaning: After loading, wash with equilibration buffer to reduce UV absorption to the appropriate value. If necessary, high salt or slightly lower pH can be added to clean the non-specific adsorption impurities.
- Elution: Use linear gradient elution method, which is 10CV equilibration solution to elution buffer (e.g. 1M sodium citrate, pH3.0). Determine the optimal pH of elution according to the peak position of antibody. If the antibody is unstable under acidic conditions, the eluent can be neutralized with a neutralizing solution (e.g. 1.0M Tris-HCl, pH9.0).

4. Application

Extrem A Diamond- Purification of a CHO expressed antibody



Column: BXP 5/20
Bed height: 10cm
Sample: CHO fermentation monoclonal antibody
Buffer solution:
A: 25mM Tris 25mM NaCl pH7.7
B: 0.5M Phosphate buffer pH6.0
C: 0.15M HAc pH2.8
D: 0.1M NaOH
Residence time: 6min



Lane 1: Original sample
Lane 2: Flow through
Lane 3: Cleaning
Lane 4: Elution dilution 10 times
Lane 5:0.1 M NaOH
Lane 6: Marker

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).

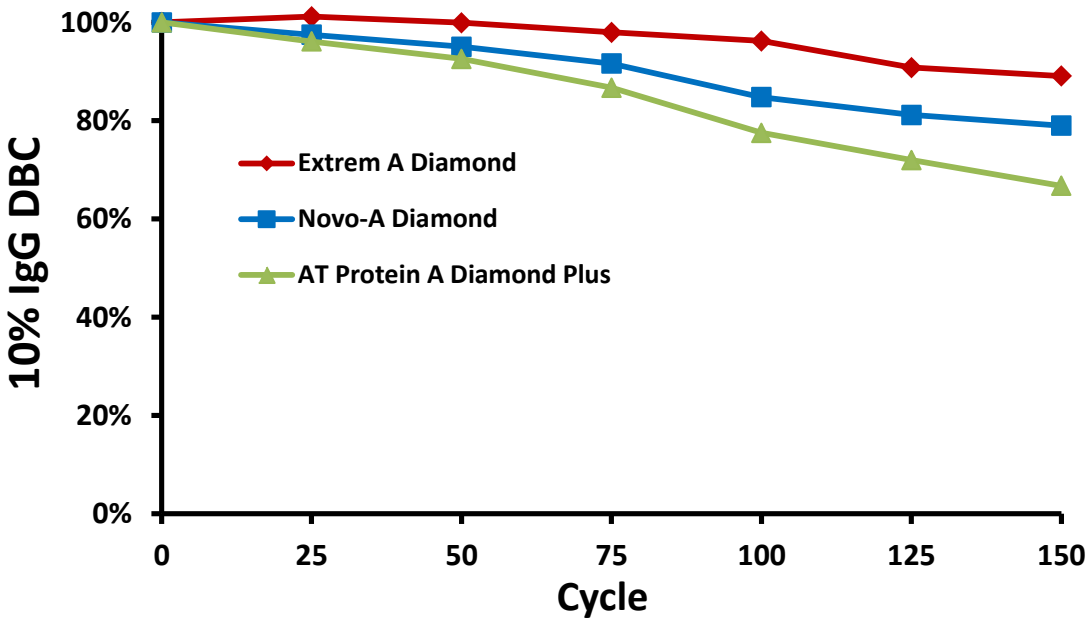
- First wash the column with 2~3CV binding buffer.
- Then wash the column with 0.1-0.5M NaOH, residence time 10-15min.

Note: Extrem A Diamond can tolerate 0.5~1.0M NaOH. NaOH concentration, residence time and contact frequency will all affect the cleaning effect. High concentration and long time can increase the CIP effect, but it will accelerate the decreasing trend of IgG binding capacity correspondingly, so generally choose 0.1~0.5M NaOH, if the pollution is serious, 0.5~1.0M NaOH can be selected.

- Immediately clean with at least 5CV binding buffer.

Note: If the antibody bound to the resin is not completely eluted, regeneration should be performed prior to CIP. Before performing CIP with NaOH, it is recommended to equilibrate the column with a neutral pH solution to avoid direct contact between the low pH buffer and the high pH NaOH solution, which may increase the temperature inside the column.

6. Alkali stability



10% IgG DBC variation after using 0.5M NaOH for CIP (Residence time 15min)

7. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have the function of sterilization and depyrogenation, it is recommended that the Extrem A Diamond resin can be treated with 0.5M NaOH for 15~30min before and during use to reduce the risk of microbial contamination and pyrogen.

8. Storage

Extrem A Diamond is supplied in 20% ethanol or 2% benzyl alcohol. It should be stored in 20% ethanol and sealed at 2-8 °C after use, in order to prevent ethanol volatilization and microbial growth, it is recommended to replace the storage solution every 3 months.

9. Disposal and recycling

Extrem A Diamond is very difficult to degrade in nature, incineration is recommended to protect the environment.

10. Order information

Product	Cat. No.	Pack size
Extrem A Diamond	AA04501	25mL
	AA04502	100mL
	AA04503	500mL
	AA04504	1L
	AA04505	5L
	AA04506	10L

Prepacked columns	Cat. No.	Pack size
EzFast Extrem A Diamond	EA04521	1×1mL
	EA04523	1×5mL
	EA04531	5×1mL
	EA04533	5×5mL
EzScreen Extrem A Diamond	EA04525	1×4.6mL
	EA04535	5×4.6mL
EzSelect Extrem A Diamond	EA04526	8×600µL
EzLoad 16/10 Extrem A Diamond	EA04501	1 pcs
EzLoad 26/10 Extrem A Diamond	EA04511	1 pcs