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AT Protein A Diamond Affinity chromatography resin

Instruction for use

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1. Introduction

AT Protein A Diamond is an innovative affinity chromatography resin made by coupling alkali-resistant Protein A with high rigidity agarose matrix through epoxy activation. Compared with traditional antibody affinity resin, the ligand enjoys better stability, faster flow velocity and lower back pressure. The resin is suitable for capturing monoclonal antibodies or Fc fusion proteins from mass cell culture media, as well as polyclonal antibodies from ascites or plasma.

The ligand of AT Protein A Diamond is the fragment of recombinant Protein A obtained by fermentation of *E.Coli*. During upstream construction, alkali-resistant amino acids were used to replace the non-alkali-resistant amino acids, while protease-sensitive amino acids were removed, providing the ligand with good alkali tolerance. The ligand fermentation and subsequent purification processes use no animal derived raw materials. The resin can tolerate CIP with 0.1~0.5M NaOH, avoiding the use of expensive and corrosive cleaning reagents, which effectively cuts costs and simplifies industrial-scale production.

2. Technical characteristics

Appearance	White slurry
Matrix	High rigidity agarose
Particle size[+]	40~120μm
Functional group	Recombinant Alkali Tolerant Protein A (24KD)
Cross-linking method	Epoxy chemistry
Dynamic binding capacity[++]	≥40mg human IgG/mL packed resin
Chemical stability	Stable in common aqueous buffers: 10mM HAc, 0.1M sodium citrate, 6M GuHCl, 8M Urea, 30%isopropyl alcohol, 20% ethanol.
Pressure flow velocity	~500cm/h,BXK300,H=20cm,<2bar
Max. pressure	0.5MPa
pH stability	3~12(working) 2~13(CIP) CIP: 0.1~0.5M NaOH
Storage[+++]	2~8℃, 20% ethanol or 2% benzyl alcohol
Recommended flow velocity	100~300cm/h

+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×1.15 (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 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 (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 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

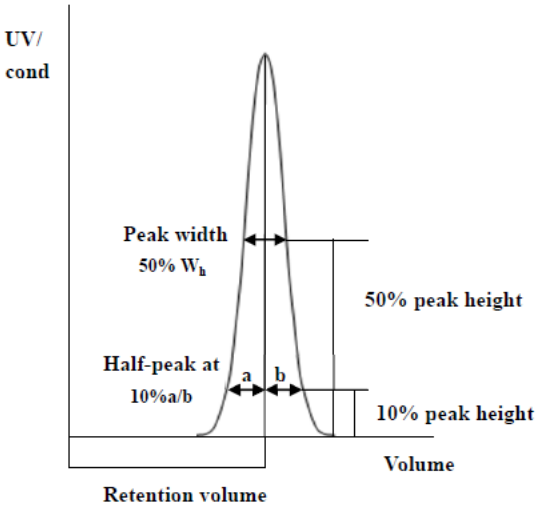
(The units of V_R and W_h should be the same)

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

- 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 between antibody and resin and reduce non-specific binding, increase pH, and 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 to avoid denaturation of unstable antibodies when the pH is too low. However, keep in mind that an overly high elution pH might compromise yield.

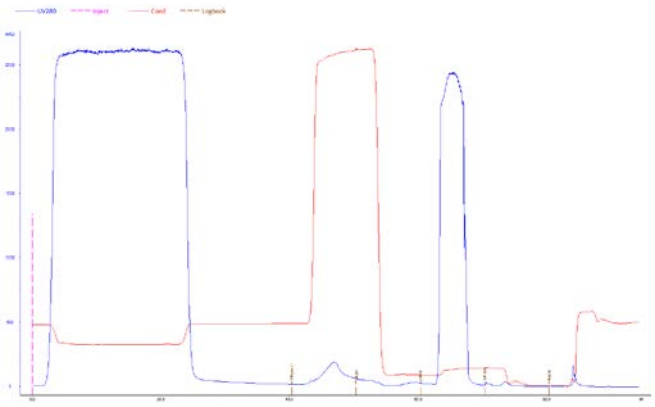
Table1. Comparison of affinity of AT Protein A for specific subtype monoclonal antibodies

Antibody	Affinity	Combine pH	Elution pH
Human			
IgG_1	Very high	6.0-7.0	3.5-4.5
IgG_2	Very high	6.0-7.0	3.5-4.5
IgG_3	Low-non	8.0-9.0	≤7.0
IgG_4	Low-high	7.0-8.0	3.0-6.0
Mouse			
IgG_1	Low	8.0-9.0	4.5-6.0
IgG_{2a}	Resin	7.0-8.0	3.5-5.5
IgG_{2b}	High	≥7.0	3.0-4.0
IgG_3	Low-High	≥7.0	3.5-5.5

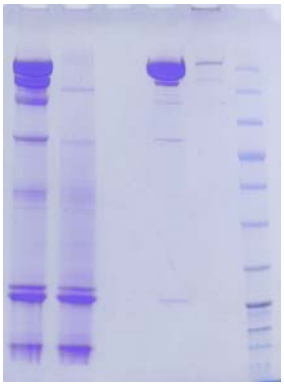
- Flow velocity: According to the height of the column, a linear flow velocity of 100 ~ 300cm/h is generally selected. The higher the column height, the slower the flow velocity.
- Sample preparation: In order to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45μm microporous membrane before loading, and the pH and conductivity of the sample are adjusted to be consistent with the equilibrium buffer. The lower the flow velocity, the higher the dynamic binding capacity.
- Equilibration: Washing the column with equilibration buffer, which usually needs 3-5CV.
- Loading sample: The loading volume is determined by the substance content and binding capacity of AT Protein A Diamond.
- Cleaning: After loading, reduce the UV absorption to the appropriate value by the equilibration buffer. If necessary, high salt or slightly lower pH can be added to clean the non-specific adsorption impurities as thoroughly as possible.
- Elution: Use linear gradient elution method, which is 10CV of equilibration buffer 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

AT Protein A Diamond purification of a CHO expressed antibody



Column: BHR5/100
Bed height: 10cm
Sample: CHO fermentation monoclonal antibody
Sample loading volume: Breakthrough capacity, $Q_{B,80\%}$
Buffer solution: A: PBS
B: 50mM NaAc, 1M NaCl pH5.5
C: 50mM NaAc pH5.5
D: 50mM NaAc pH3.6
E: 1M HAc
F: 0.1M NaOH
Flow velocity: 0.33mL/min



1 2 3 4 5

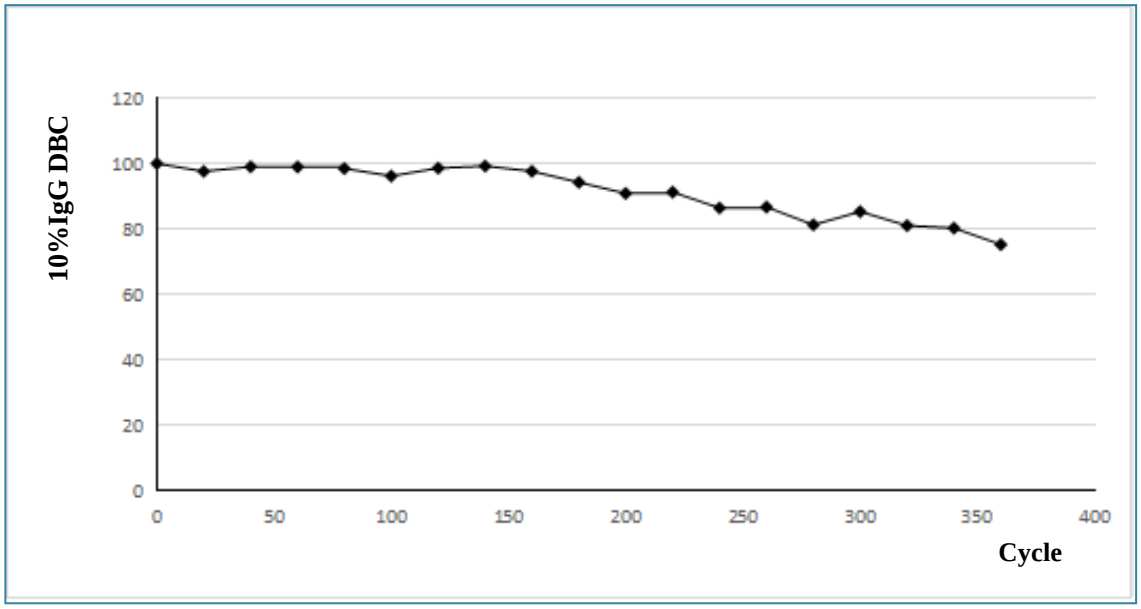
Lane 1: Original sample
Lane 2: Flow through
Lane 3: Cleaning
Lane 4: Elution
Lane 5:0.1 M NaOH to wash

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).

- Wash with 2CV of 2M NaCl to remove strong non-specifically bound proteins.
- Then wash the column with 0.1-0.5M NaOH, residence time 10-15min.
- Immediately with at least 5CV of binding buffer.

6. Alkaline stability



AT Protein A Diamond with 0.1M NaOH CIP (residence time: 33.52min) for 10%IgG dynamic load.

7. Sterilization

Since 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation functions, it is recommended that AT Protein A Diamond resin can be treated with 0.1M NaOH for 30min or 0.5M NaOH for 15min to reduce the risk of microbial contamination before and during use.

8. Storage

AT Protein A Diamond is supplied in 20% ethanol or 2% benzyl alcohol. It should be stored in 20% ethanol and sealed at 2-8℃ 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

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

10. Order information

Product	Cat. No.	Pack size
AT Protein A Diamond	AA0272	25mL
	AA0273	100mL
	AA0274	500mL
	AA0275	1L
	AA0276	5L
	AA0277	10L
	AA302315	20L

Prepacked columns	Cat. No.	Pack size
EzFast AT Protein A Diamond	EA302301	1×1mL
	EA302303	1×5mL
	EA302351	5×1mL
	EA302353	5×5mL
EzScreen AT Protein A Diamond	EA02725	1×4.6mL
	EA02735	5×4.6mL
EzSelect AT Protein A Diamond	EA02726	8×600µL
EzLoad 16/10 AT Protein A Diamond	EA302304	1 pcs
EzLoad 26/10 AT Protein A Diamond	EA302306	1 pcs