BESTCHROM
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Diamond Protein L Affinity chromatography resin

Instruction for use

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1、Introduction

Diamond Protein L is an innovative affinity resin obtained by coupling Protein L ligands on the highly rigid agarose base matrix via epoxy activation. Its highly rigid agarose enjoys higher flow velocity and lower back pressure than conventional resins. Besides, the variable zone between the Protein L ligands and Kappa antibody light chain possesses strong affinity. It is also suitable for the capture of antibody fragments, including Fab, single chain antibody fragment(scFv) and regional antibody(dAbs), from large-volume cell culture media.

Advantages：

- High rigid agarose base matrix, enabling high flow velocity and improved productivity.
- Highly specific Kappa light chain, enabling efficient capture of antibody and antibody fragments with wide selection.
- High binding capacity, reduce production time and resin consumption, lower cost and improved productivity.

2、Technical Parameters

Appearance	White slurry, can be layered
Matrix	High rigid agarose
Average particle size+	~80μm
Functional group	Recombinant Protein L(from *E.coli*)
Cross-linking method	Epoxy chemistry
Dynamic binding capacity++	~40 mg Human IgG/mL packed resin（Retention time 6min）
Chemical stability	Common aqueous solutions：10mM HCl、0.1M citric acid （pH3）、6M Urea、6M GuHCl、30% isopropanol、20%ethanol
Max. pressure	0.5MPa
Pressure flow velocity	≥300cm/h，<2bar，BXK300/500，H=20cm
pH stability	2~10（working），15mM NaOH（CIP）
Storage+++	2~8℃，20% ethanol or 2% benzyl alcohol

+ Particle size is normally distributed, average particle size is the medium value of the particle size in resin

++Flow velocity 100cm/h，bed height 10cm，Buffer solution：20mM PB、0.15M NaCl，pH7.2

+++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 settlement resin required, the suspended slurry of the resin required is calculated by the follow:
  Required resin slurry1 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 (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 glass tube 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: 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 50cm/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
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

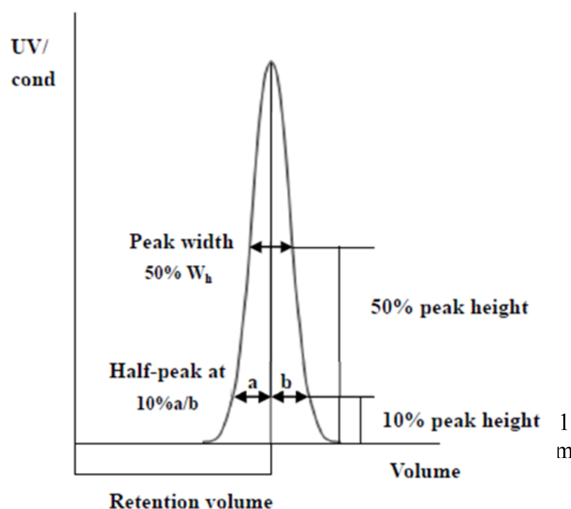
  W_h = half-peak width

  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

- Buffer: Normally, use neutral buffers as the binding buffer (20mM PB 0.15M NaCl pH7.2). Use buffers with low pH for elution (0.1M Sodium citrate pH2.0-3.5). When optimizing elution condition, the max pH still needs to be explored to prevent the degeneration of unstable antibodies at low pH value.
- 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 4-6 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 equilibrium buffer.
- Sampling: The loading volume is determined according to the substance content and binding load of Diamond Protein L.
- Wash： Wash with ~5CV binding buffer to lower the UV at an appropriate rate. If necessary, high salt or slightly lower pH can be added to clean the non-specific adsorption impurities as much as possible.
- Elution: A linear gradient of 10CV from equilibrium solution to elution buffer (e.g. 1M sodium citrate, pH3.0) can be used to 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).
- Regeneration：Wash the column with 5~10CV elution buffer solution.

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).

- First wash the column with 2~3CV binding buffer.
- Then use 15mM NaOH to clean ~3CV, contact time 10~15min;
- Wash with binding buffer （pH7~8）for at least 5CV.

Note： Regeneration should be done before CIP if antibodies on resin are not sufficiently eluted. It

is recommend to balance the column with neutral pH buffer before CIP with NaOH. Never let low pH buffer directly being exposed to high pH NaOH solution to prevent temperature rise in column.

5、Storage

Diamond Protein L 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.

6、Disposal and Recycling

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

7、Order information

Product	Code No.	Pack size
Diamond Protein L	AA05101	25mL
	AA05102	100mL
	AA05103	500mL
	AA05104	1L
	AA05105	5L
	AA05106	10L

Prepacked columns	Code No.	Pack size
EzFast Diamond Protein L	EA05121	1×1mL
	EA05123	1×5mL
	EA05131	5×1mL
	EA05133	5×5mL
EzScreen Diamond Protein L	EA05125	1×4.9mL
	EA05135	5×4.9mL
EzLoad 16/10 Diamond Protein L	EA05101	1 pcs
EzLoad 26/10 Diamond Protein L	EA05111	1 Pcs