BESTCHROM
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Protein G Bestarose 4FF Affinity chromatography resin

Instruction for use

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1. Introduction

Protein G Bestarose 4FF is an affinity resin made by fixing Protein G to Bestarose 4FF base frame by cyanogen bromide activation. Protein G has a broader binding spectrum than Protein A. At the same time, Protein G not only has strong binding to the Fc fragment of the antibody, but also has weak interaction with the Fab fragment of the antibody, so Protein G Bestarose 4FF is often used for separation Purification of antibodies or antibody fragments from cell culture media and purification of antibodies from sera of various species.

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	Highly cross-linked agarose, 4%
Particle size+	45-165μm
Functional group	Protein G(*E.coli*)
Ligand concentration	~2mg Protein G /mL resin
Cross-linking method	Cyanogen bromide activation
Dynamic binding capacity++	~20mg human IgG/mL packed resin
Chemical stability	40 ℃ ,1 week: 1M HAc(pH2.0),20 mM sodium phosphate , 1%SDS, 6M GuHCl, 70% ethanol At room temperature 2h：0.1 M HCl (pH1.0), 8M Urea (pH10.5)
Physical stability	Volume changes due to changes in pH or ionic strength are negligible
Max. pressure	0.3MPa
Pressure flow velocity	150~250cm/h（0.1MPa BXK50 H=25cm）
pH stability	3~9(working)　2~10 (CIP)
Storage+++	2~8℃,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

++ Linear flow velocity 200cm / h, bed height 10cm, buffer conditions: 20mM PB, 0.15M NaCl, pH 7.4

+++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 (20% ethanol)/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 45cm/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), Install the adaptor lower the adaptor to about 0.5cm above the resin surface, set the flow velocity at 160cm/h, 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

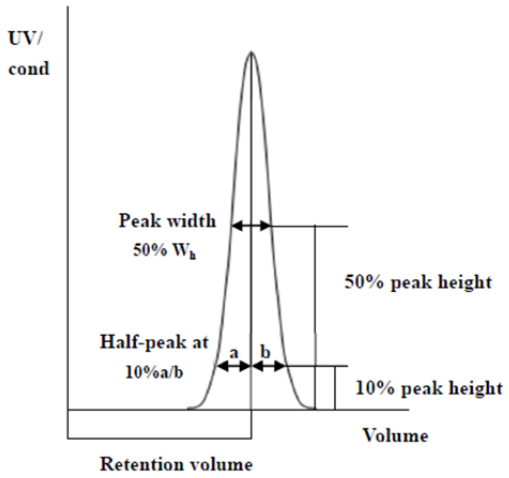
  (The units of V_R and W_h should be the same)

  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

- Buffer： Protein G binds to different types of IgG in a neutral environment. It is recommended to use 20mM PB, pH 7.0 as the binding buffer.
- Comparison of the binding ability of Protein A and Protein G with different immunoglobulins (Table 1).

Table 1. Comparison of protein A and Protein G binding strength with different types of immunoglobulins

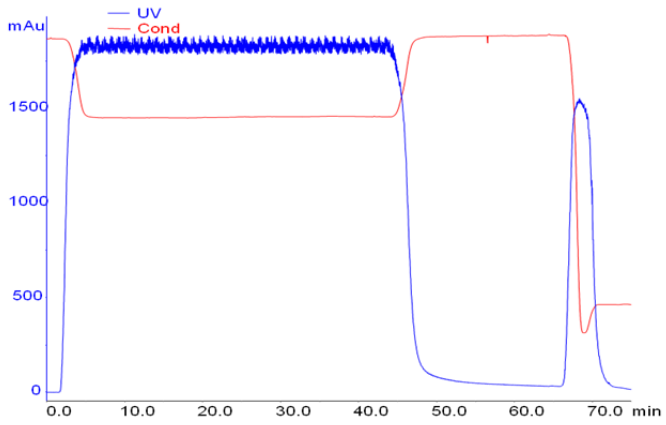
Species	Subtype	Combination strength with Protein G	Combination strength with Protein A
Human	IgA	-*	Variable
	IgD	-	-
	IgE	-	-
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	++++	-
	IgG_4	++++	++++
	IgM	-	Variable
Bird yolk	IgY	-	-
Cattle		++++	++
Dog		+	++
Goat		++	-
Guinea pig	IgG_1	++	++++
Big-cheeked rodent		++	+
Horse		++++	++
Treekangaroo		+	-
Camel		+	-
Rhesus Monkey		++++	++++
Mouse	IgG_1	++++	+
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	+++	++
	IgM	-	Variable
Pig		+++	+++
Rabbit		+++	++++
Rat	IgG_1	+	-
	IgG_{2a}	++++	-
	IgG_{2b}	++	-
	IgG_3	++	+
Sheep		++	+/-

* More plus signs indicate stronger bonding strength, minus signs indicate weaker bonding strength or even no bonding

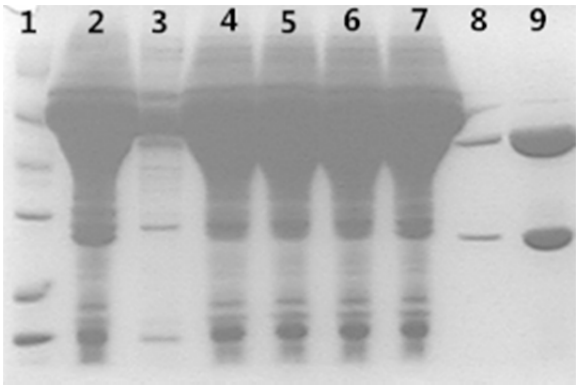
- Flow velocity: The flow velocity of 50~300cm/h is generally selected according to the height of the column. The higher the column height is, the slower the flow velocity is. Reducing the flow velocity can increase the residence time between the sample and the resin and improve the binding load.
- Sample 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.
- Balance: Wash the column with a balance buffer until uv absorption is reduced to appropriate values.
- Sampling: Sample the prepared sample solution according to the set conditions.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution: Usually change the buffer pH for elution, it is recommended to use 0.1M glycine pH 2.5-3.0 as the elution buffer. In order to maintain the activity of certain acid-sensitive IgG, the elution collection tube can be pre-added with 1/10 of the collection volume of 1M Tris-HCl, pH 9.0 for neutralization.
- Regeneration: After elution, the resin should be cleaned with 2-3CV of eluting buffer immediately, and then balanced with 2-3CV of binding buffer. For example, denatured proteins or lipids in the process can not be elute in the regeneration process, and can be removed by in-place cleaning operation.

4. Application

Protein G Bestarose 4FF purified mouse IgG



Column: Ezload 16/10 Protein G 4FF
Buffer A: PBS
Buffer A: 0.1M Gly,pH2.7
Sample: rat ascites sample



Lane1:Marker
Lane2:Ascites in mice
Lane3:Flow through
Lane4:Flow through
Lane5:Flow through
Lane6:Flow through
Lane7:Flow through
Lane8:Elution dilute 10 times
Lane9:Elution

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).

- It can be soaked with 70% ethanol for 12 hours to remove lipid substances, and then rinsed with at least 5CV of binding solution.
- It can be treated with a non-ionic detergent such as 0.1% Trtion X-100 at 37℃ with a residence time of 1 min, and then quickly rinsed with at least 5CV of binding solution.

6. Sterilization

Since the 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and depyrogenation, it is recommended that Protein G Bestarose 4FF can be treated with 70% ethanol for more than 12h to reduce the risk of microbial contamination before and during use.

7. Storage

Protein G Bestarose 4FF 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.

8. Disposal and Recycling

Protein G Bestarose 4FF is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Code No.	Pack size
Protein G Bestarose 4FF	AA0142	25mL
	AA104307	100mL
	AA104311	500mL
	AA0144	1L
	AA0145	5L
	AA104314	10L

Prepacked columns	Code No.	Pack size
EzFast Protein G 4FF	EA104301	1×1mL
	EA031	1×5mL
	EA032	5×1mL
	EA033	5×5mL
EzScreen Protein G 4FF	EA01425	1×4.9mL
	EA01435	5×4.9mL
EzLoad 16/10 Protein G 4FF	EA104304	1 pcs
EzLoad 26/10 Protein G 4FF	EA104306	1 pcs