BESTCHROM
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GST Bestarose 4FF Affinity chromatography resin

Instruction for use

Contents

1. Introduction

GST Bestarose 4FF is made by coupling glutathione to a highly cross-linked agarose gel, which is specially used to specifically purify glutathione S-Transferase (GST) and GST fusion protein Separation and purification. GST tag is a tag commonly used in modern genetic engineering to express fusion proteins, which is conducive to the soluble expression and activity maintenance of proteins. For different sources of glutathione S-transferase and its fusion proteins, this resin can be purified in one step High-purity target protein can be obtained. The chromatographic resin has high pressure resistance, fast flow velocity, and mild operating conditions, which is conducive to the maintenance of protein activity.

2.Technical characteristics

Appearance	White slurry, can be layered
Matrix	Highly cross-linked agarose, 4%
Particle size+	45-165μm
Functional group	Glutathione with 10 atomic arms
Ligand concentration	120~320μmol Glutathione /mL resin
Dynamic binding capacity	~10mg GST/mL packed resin
Max. pressure	0.3 MPa
Chemical Stability	Stable in common aqueous buffers: 1M HAc++, 70%ethanol, 6M GuHCl (1 hour at room temperature)
pH stability	3~12
Storage+++	2~30℃，20%ethanol or 2% benzyl alcohol
Pressure flow velocity	~450cm/h，BXK16/10，H=5cm，25℃
Recommended flow velocity	Sample loading flow velocity：＜100cm/h Balance, cleaning, elution flow velocity：100~300cm/h

+Particle size is normally distributed, and particles within this range account for more than 95% of the total.

++1M HAc only be used for cleaning.

+++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 (PBS,pH7.3)/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 450cm/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
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

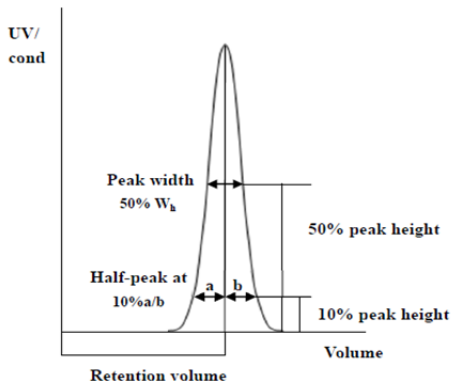
  L = column height

  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.

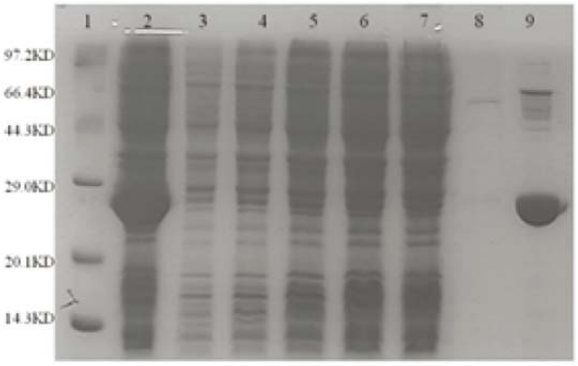
3.3 Chromatographic method

- Sample solution
  - In order to avoid clogging the chromatography column, the sample solution needs to be centrifuged or filtered with a 0.45μm filter.
  - The viscosity of the sample needs to be appropriate. High viscosity samples will cause uneven flow velocity during the chromatography process and affect the mass transfer balance.
- Binding buffer：Generally, a neutral buffer solution is used, such as 20 mM PB, 0.15 M NaCl, pH 7.3.
- Flow velocity：According to the height of the column, the flow velocity of <100cm / h is generally selected, and the low flow velocity is conducive to protein binding.
- Sample preparation: The pH and conductivity of the sample are adjusted to be consistent with the equilibration buffer, and determine the loading volume according to the binding capacity of the resin and the content of the target substance in the sample.
- Equilibrium: Wash the column with a binding buffer until the UV absorption is reduced to an appropriate value.
- Sampling: Sample loading shall be carried out according to the set conditions.
- Rinse: Wash the column with equilibration buffer until the UV absorption value is reduced to an appropriate value.
- Elution：Commonly used reduced glutathione for elution, such as: 50mM Tris, 10mM reduced glutathione, pH8.0.

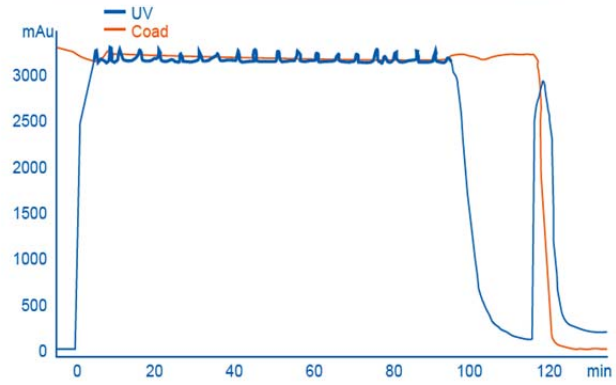
  1-10mM DTT can be added to the buffer to increase the purity of the target.
- Regeneration: 2CV of high pH buffer (0.1MTris-HCl, 0.5MNaCl, pH8.5) and low pH buffer (0.1M sodium acetate, 0.5M NaCl, pH4.5) were washed alternately for three times.10CV combined with buffer balanced chromatography column.

4. Application

The recombinant GST label protein expression solution was purified by GST Bestarose 4FF



Column：EzFast 5mL GST 4FF
Buffer A：0.15M NaCl、20mM PB， pH7.4
Buffer B：15mM reduced glutathione, 50mM Tris, pH8.0
Sample: Recombinant GST label protein expression solution



Some sample order（DTT reduction electrophoresis）:
Lane1：Marker
Lane2：Sample
Lane3-7：Flow through
Lane8：Rinse
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).

The recommended CIP for different types of impurities and contaminants are as follows:

- Precipitated or denatured substances:
  Wash with 2CV of 6M GuHCl followed by 5CV of equilibrationbuffer.
- Hydrophobic binding substance:
  Wash with 2~4CV of 70% ethanol, then wash with 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 GST Bestarose 4FF can be treated with 70% ethanol for more than 12h to achieve the purpose of sterilization and depyrogenation.

7. Storage

GST Bestarose 4FF 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 every 3 months.

8. Disposal and Recycling

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

9. Order information

Product	Code No.	Pack size
GST Bestarose 4FF	AA0071	25mL
	AA0072	100mL
	AA0073	500mL
	AA0074	1L
	AA0075	5L
	AA109314	10L

Prepacked columns	Code No.	Pack size
EzFast GST 4FF	EA109301	1×1mL
	EA109303	1×5mL
	EA012	5×1mL
	EA013	5×5mL
EzScreen GST 4FF	EA00725	1×4.9mL
	EA00735	5×4.9mL
EzLoad 16/10 GST 4FF	EA109304	1 pcs
EzLoad 26/10 GST 4FF	EA109306	1 pcs