BESTCHROM
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Chromdex S-100
Chromdex S-200
Gel Filtration Resin
Instruction for use

Contents

1. Introduction

Chromdex S-100、Chromdex S-200 are based on the highly cross-linked agarose and filled with cross-linked dextran. The resin possesses high selectivity of dextran and better mechanical property of agarose. It can be an ideal choice for fine purification with advantages including, high resolution, high rigidity, high flow velocity and minimal bed volatility in various buffer concentrations, better chemical stability, lower non-specific adsorption, high yield and easy scale-up.

2. Technical characteristics

Product		Chromdex S-100	Chromdex S-200
Chemical component		Highly cross-linked agarose and dextran	
Fractionation range	Globular molecule	1KD~100KD	5KD~250KD
	Dextran	/	1KD~80KD
Appearance		White slurry, can be layered	
Particle size+		25~75μm	
Pressure flow velocity		>125cm/h（0.1MPa BXK50/100,H=15cm）	
Max. pressure		0.3MPa	
pH stability		3~12（working）,1~14（CIP）	
Chemical stability		Stable in common aqueous buffers：0.5M NaOH++、0.1M HCl、1M HAc、8M Urea、6M GuHCl、70% ethanol、30% isoprophanol、1%SDS、30% acetonitrile、2M NaCl, etc.	
Storage+++		2~30℃, 20% ethanol or 2% benzyl alcohol	

+ Particle size is normally distributed, and particles within this range account for more than 85% of the total

++0.5M NaOH 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 recommended to equilibrate the resin slurry to room temperature before column packing.

- Resin calculation：Calculate the amount of Chromdex resin needed according to the CV used
  Resin volume=column volume×1.15（Compression factor=1.15）
  Calculate the slurry needed by the suspended resin volume：

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(%)
100mL、500mL、1L、5L、10L	80
20L、40L	75

1: It refers to the original packaged resin slurry sold by Bestchrom.

Note： For resin in non-original concentration, customer can calculate the required volume according to the actual concentration of resin slurry.

- Washing the resin: Thoroughly shake the resin and weigh certain volume of resin calculated by the method mentioned above. Pour it into a funnel, drain the liquid, and wash with about 3mL distilled water/mL resin for 3 times. Use a glass stick or stirrer to stir each time when adding distilled water, which helps to wash the shipping solvent away.
- Prepare the packing slurry: Transfer the washed resin from the funnel into a beaker or other appropriate container, add distilled water 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 BXK16/70 for example, purge the bubbles trapped at the end-piece net by draining some distilled water 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 packing reservoir 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.

Note: This step is only applicable for BXK50 and smaller columns.

✧ **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 60cm, the flow velocity can be set to 35cm/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, set the flow velocity at 380cm/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

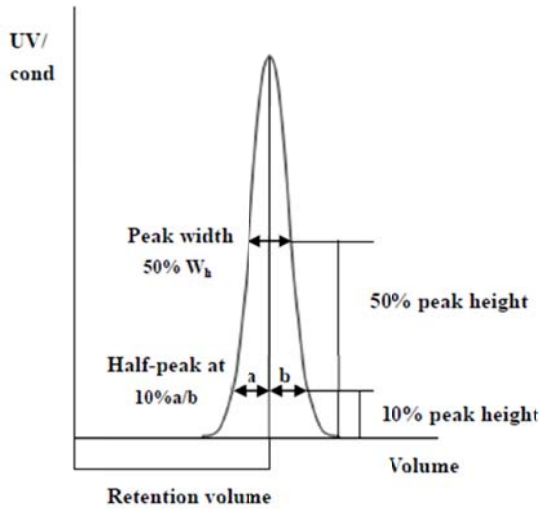
  (The units of V_R and W_h should be the same)

  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 0.7~1.3, the column is very efficient. (For Chromdex resin, the number of

trays per meter should be greater than 7,000) .The unsatisfactory results should be analyzed and the column should be repacked.

3.3 Chromatography method

- Equilibrium: Wash the column with equilibrium buffer at the recommended flow velocity. Choose buffer according to the sample stability since buffer type and pH value have very little impact on the resin performance. However, Due to the fact that agarose contains tiny amount of sulfate and carboxyl groups, it is recommended to add a minimal 0.15M NaCl to the buffer to reduce the non-specific adsorption of basic protein samples. The equilibrium is regarded as completed when the PH and conductivity of buffer outflow are same as these of the buffer inflow, which will take 2-3 CV of buffer.
- Sample preparation: To avoid clogging the column, 0.45μm membrane filtration is needed before loading sample.
- Load sample: Load sample by sample ring, the load volume might vary from target types and impurity types. Normally, load 1-4% CV of sample and adjust the sample volume according to separation performance.
- Separation: Wash column with equilibrium buffer and collect different components till no bio-molecules flow out. This process will usually take 1-1.5 CV.
- Regeneration: Equilibrate the column with 2-3 CVs of buffer.
- Re-equilibrium: Wash column with buffer till the pH and conductivity rate reach the same with buffer. Load sample again and repeat the above-mentioned process.

4. Cleaning-in-place (CIP)

After being used for a long time period, Chromdex S-100、Chromdex S-200 will suffer from reduced column efficiency, high back pressure, poor separation and colour-change of resin. CIP(it is recommended to be performed every 5 cycles) can be done according to the following procedure:

- Wash with 1CV of buffer+1M NaCl
- Remove the denatured protein
  - Reversely wash with0.5M NaOH at20-25cm/h for 2CVs.

  Note：Denatured protein can be removed by protease. Use 1 mg/mL of gastric enzyme dissolved in 0.1 M acetic acid solution +0.5 M NaCl.
- Remove the lipids and lipoprotein
  - Wash with 4CV of 70% ethanol or isopropanol at 30cm/h. (To avoid air bubble, gradient increase the proportion of organic solutions).
  - Alternatively, use 1% non-ionic detergent.
- Inorganic pollutants
  - Wash with 2CV of 0.5M acetic acid
- Wash with 4CV of distilled water

5. Sterilization

Chromdex S-100、Chromdex S-200 are stored in 20% ethanol or 2% benzyl alcohol. Since both storage solutions have no sterilization or depyrogenation function. To reduce microbial contamination, wash with 0.5-1 M NaOH at room temperate for 0.5-1h or wash column for 1-2h prior to the use of resin.

6. Storage

Chromdex S-100、Chromdex S-200 are stored in 20% ethanol or 2% benzyl alcohol. The unsealed resin should be preserved in 20% ethanol at 2~30℃. it is recommended to replace the storage solution every 3 months to prevent ethanol volatilization and microbial growth.

7. Disposal and Recycling

Chromdex S-100、Chromdex S-200 are very difficult to degrade in nature, incineration is recommended to protect the environment.

8. Order Information

Product	Code No.	Pack size
Chromdex S-100	AG322107	100mL
	AG322111	500mL
	AG322112	1L
	AG322113	5L
	AG322114	10L
	AG322115	20L
	AG322116	40L

Prepacked columns	Code No.	Pack size
EzLoad 26/90 Chromdex S-100	EG02313	1 pcs